

The
National
Eye
Institute



Annual Report

Fiscal Year
1980

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THE NATIONAL EYE INSTITUTE.

ANNUAL REPORT of program activities.

Fiscal Year 1980

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STATEMENT OF THE INSTITUTE DIRECTOR

During its first decade the National Eye Institute focused its attention on developing and strengthening the research base in the visual sciences. In FY 1980, this continued to be our primary concern, but we also began exploring new ways of fostering the clinical application of research results with the aim of improving eye care and more effectively preventing or alleviating eye disease and blindness.

Knowledge Transfer

To provide a focal point within the Institute for identifying knowledge transfer opportunities and for recommending specific strategies to exploit them, an Office of Clinical Applications of Vision Research was established. In carrying out these responsibilities, the Office works closely with other NEI components that have knowledge transfer functions.

For example, the new Office collaborated with the NEI Information Office which was in the process of planning a campaign to encourage eye care specialists, primary care physicians, and allied health workers to apply the results of the Diabetic Retinopathy Study of photocoagulation to the routine care of diabetic patients. A plan for a multimedia campaign to disseminate information on diabetic retinopathy to the press and general public was also developed with the objective of increasing public awareness of the ocular complications of diabetes and their treatment.

Radial Keratotomy

It is noteworthy that this knowledge transfer effort is based upon the results of a successful controlled clinical trial, for the NEI's position is that before new methods of treating and diagnosing visual disorders are routinely incorporated into clinical practice, they should first be subjected to rigorous scientific evaluation. It is for this reason that the National Eye Institute and the National Advisory Eye Council have become increasingly concerned by the rapid adoption in the United States of radial keratotomy, a new surgical technique for correcting myopia. The Institute and the Council believe that to date the benefits and risks of this procedure have not been adequately assessed. At its January 1979 meeting, the Council called for more animal research on refractive keratoplasty, of which radial keratotomy is one form. The Council also took the position at that time that the NEI should not support clinical research on these procedures until the results of animal studies could be evaluated.

Nevertheless, it soon became evident that as a result of extensive national publicity favorable to radial keratotomy there might soon be a great public demand for this procedure; in fact, increasing numbers of ophthalmic surgeons were already performing the operation. Therefore, at its May 1980 meeting the Council took the unprecedented step of passing a resolution expressing its concern over the increasing use of radial keratotomy in the absence of adequate scientific data on its benefits and risks. The

Council urged eye care professionals and the public to exercise restraint in utilizing this procedure until the results of clinical studies could be assessed. The resolution, which also called for more research on radial keratotomy, was released to the news media and subsequently received widespread coverage in the press and on radio and television. The American Academy of Ophthalmology and several other national and local ophthalmological organizations have endorsed the Council's statement.

When in July a group of ophthalmologists submitted a research grant proposal for a cooperative controlled clinical trial of radial keratotomy, the NEI immediately arranged for scientific merit review of the group's application so that it could be considered by the National Advisory Eye Council at its September meeting. The Council approved the study, and initial NEI funding is being provided. The investigators are now working closely with the NEI staff to prepare a final draft of the study's manual of procedures.

The rapid and widespread introduction and acceptance of radial keratotomy as a treatment for myopia underscores the need for the NEI and the Council to identify early important public interest issues that may be inherent in emerging medical technologies and to take whatever action on these questions may be appropriate after consultation with leading representatives of the scientific and practitioner communities.

Scientific Workshops

Another aspect of knowledge transfer is to foster interchange between vision scientists and those in other fields of biomedicine. To this end, several scientific meetings were sponsored during the year. Especially noteworthy and successful were a series of workshops held in response to the recommendation made in the National Advisory Eye Council's report Vision Research--A National Plan: 1978-1982 for more research on the immunological aspects of ocular diseases and for the application of new knowledge in the field of immunology to study of the visual system. These workshops were planned in cooperation with the National Institute of Allergy and Infectious Diseases to stimulate discussion and collaboration among vision researchers and immunologists.

The first workshop in the series, "Immunogenetics and Transplantation Immunity," was held December 5-7, 1979, and led to the identification of several ways in which recent advances in immunogenetics and transplantation immunology might be applied productively in eye research. A second workshop, entitled "Autoimmune Phenomena and Ocular Disorders," was convened March 5-7, 1980, and identified promising avenues for research on autoimmunity in relation to specific ocular diseases. The third and final workshop in this series, "Infection, Inflammation, and Allergy," was held June 25-27. Participants at this meeting discussed possible ways to minimize the destructive effects of inflammation associated with ocular diseases while maximizing the protective effects of the inflammatory process.

The proceedings of all three workshops are being published as special supplements to the journal Immunology Abstracts and will be widely disseminated. However, we have already begun to see the fruits of this collaborative effort in the submission of grant applications for research in some of the high priority areas identified by workshop participants.

Because of a continuing controversy in the scientific literature concerning the management of ocular melanoma, the NEI convened a task force of experts from the fields of ophthalmology, oncology, and epidemiology to identify the most important research and clinical questions in this important field and to recommend research initiatives that the NEI should pursue. Following an evaluation of the recent literature on the subject, the task force made a number of recommendations for additional research on the epidemiology, biology, diagnosis, pathology, and natural history of ocular melanoma, including a randomized, controlled clinical trial of alternative methods of ocular melanoma treatment. The task force's recommendations will be published in a forthcoming edition of the American Journal of Ophthalmology, and the NEI will soon issue a program announcement soliciting grant applications in response to them.

Clinical Trials

During FY 1980, the NEI provided support for a new clinical trial that is comparing three forms of cataract treatment: two forms of cataract extraction followed by intraocular lens implantation and cataract surgery followed by the use of contact lenses. This trial will compare the interim and long-term visual acuity of patients in these three treatment groups, document the extent and rate of development of complications, and identify factors that can be used to predict final visual results. The initiation of this study brings to 14 the number of clinical trials now being supported by the NEI. Some of these are large-scale, multicenter, cooperative studies; others involve only one center. The NEI uses both contracts and grants to support these studies, several of which are being conducted by investigators who first became involved in clinical trials by participating in the cooperative Diabetic Retinopathy Study.

As a further means of increasing interest in clinical research and helping to expand the manpower base available for designing and conducting clinical trials, the National Eye Institute presented a course in June on "Clinical Vision Research: Epidemiologic and Biostatistical Approaches," in cooperation with the Dartmouth-Hitchcock Medical Center. This course was well-attended and enthusiastically received, and it will be repeated during FY 1981.

International Research

Although the NEI's primary mission has been and will continue to be to support research on eye problems common in the United States, we have also been alert to research opportunities in other nations, not only for the study of problems we share but also of those which though uncommon here account for a major share of the world's blindness and visual disability. Because

the number of blind people throughout the world is now at least 40 million, the National Eye Institute cannot ignore its responsibility to assist health professionals in developing nations establish a research base upon which programs aimed at reducing the alarming incidence and prevalence of global blindness can be designed.

During the past year, we worked with the World Health Organization on programs for the prevention of blindness in Africa. Initially, attention will focus on Mali and the Sudan where epidemiological surveys will be carried out and blindness prevention programs established. In addition, projects aimed at improving existing trachoma control programs are underway in Egypt, Tunisia, and Morocco with support from the NEI and the P.L. 480 program.

These NEI efforts in Africa may presage a greater role for NIH generally on this continent. On a recent visit to four countries in Africa with the President's Science Advisor, the NIH Director identified many scientific opportunities in these nations.

We are also pursuing ways of providing assistance in India, where cataract and nutritional blindness take a great toll. Nutritional deficiency, primarily vitamin A deficiency, causes blindness in an estimated 20,000 Indian children each year. The NEI is studying the possibility of establishing a multidisciplinary research center for the study of nutritional blindness in Hyderabad, in cooperation with the Indian National Institute of Nutrition. The NEI has also advised on the design of a study to evaluate the most effective means of providing services to cataract patients in India through the use of mobile units. Although only a very small portion of NEI's financial and manpower resources are being committed to these international activities, their potential worldwide benefits are great.

Other Developments in FY 1980

Other significant events during the year included the National Advisory Eye Council's passing a resolution expressing concern over the increasing portion of NIH funds that must be allocated to support indirect institutional costs of research. The Council noted that at the present time, about one-third of NEI extramural research funds are spent for such indirect cost allowances. Although the Council acknowledged the complexity of this issue and recognized it as one that transcends the expertise and authority of the National Eye Institute, its action, which was widely reported in the scientific press, helped direct the attention of the scientific community at large to the question of increasing indirect costs. It is now a major topic of debate and study at higher levels of government and within the academic community.

NEI's new vision research facilities construction program was announced in September in the NIH Guide for Grants and Contracts, following NIH approval of the program's guidelines. A total of \$3 million is available for this program in which the NEI will provide up to 50 percent of the total allowable cost of a project up to a maximum of \$500,000. The deadline for applications is May 1, 1981.

Notable among honors bestowed on members of the NEI staff during the past year was an honorary Doctor of Science awarded to Dr. Jin H. Kinoshita,

Acting Scientific Director, by Oakland University in Rochester, Michigan. Dr. Kinoshita also delivered the commencement address at the University in June. Drs. Gerald J. Chader and Leo Liu of the NEI Laboratory of Vision Research shared in a citation for outstanding achievement in basic research awarded by Fight for Sight, Inc., a national philanthropic organization. Also cited were Dr. Gopal Krishna of the National Heart, Lung, and Blood Institute and Dr. Gustavo Aguirre of the University of Pennsylvania School of Veterinary Medicine. The four investigators were honored for their research on "Cyclic GMP Phosphodiesterase Activator: Involvement in a Hereditary Retinal Degeneration." This award-winning study of enzyme activity provides clues to the development of a treatment for certain types of retinitis pigmentosa in humans.

Highlights of other advances in research during FY 1980 in the NEI's extramural and intramural programs and reports of important administrative developments during the past year may be found in the following pages.



Carl Kupfer, M.D.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1979 - September 30, 1980

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS
Ronald G. Geller, Ph.D.

In keeping with the Institute's first priority, support for investigator-initiated individual research projects (ROI), almost 900 awards were made covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. This represents about 90 percent of our extramural budget. The following sections highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1980.

For FY 1980, the National Eye Institute received an appropriation of \$113,000,000--an increase of \$7,808,000 over the previous year's appropriation. Of the \$113,000,000, a total of \$95,644,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Research Grants	\$85,560,000
Research Training Awards	4,593,000
Research Contracts	<u>5,491,000</u>
Total	<u>\$95,644,000</u>

These funds were distributed among the Institute's five programs as follows:

	Research, Training, & Contract Dollars (in thousands)
Retinal and Choroidal Diseases	\$42,162
Corneal Diseases	14,163
Cataract	9,085
Glaucoma	10,355
Sensory and Motor Disorders of Vision and Rehabilitation	<u>19,929</u>
Total	<u>\$95,644</u>

The grant application receipt rate was slightly less than in FY 1979. The National Advisory Eye Council approval rate, however, was stable during these two fiscal years: 85 percent of grants submitted were approved for funding in both FY 1979 and 1980. The Institute was able to fund only 47 percent of all approved applications, considerably lower than in FY 1979. The data are given below:

	<u>Grant Application Rate¹</u>			
	<u>Received & Reviewed</u>	<u>Recommended for Approval</u>	<u>Approved & Funded</u>	<u>% Funded of All Approved Applications</u>
FY 1978	681	562	343	61
FY 1979 ²	579	495	308	62
FY 1980 ²	516	432	201	47

¹ R01 and R23.

² FY 1980 figures are estimates based on current information.

The distribution of awards (for R01s and R23s) between competing and noncompeting research grant applications was as follows:

	<u>FY 1978 Number of Grants</u>	<u>FY 1979 Number of Grants</u>	<u>FY 1980 Number of Grants</u>
Prior Year Commitments	434	577	679
New Research Awards	225	177	86
Renewal Awards	<u>110</u>	<u>115</u>	<u>115</u>
	769	869	880

The Institute's research grants are comprised of the following categories:

FY 1980 Research Grants by Mechanism
(Dollars in Thousands)

	<u>Number</u>	<u>Total Awarded</u>
Research Grants (R01), R10, R13, R23)	941	34,950
Core Center Grants (P30)	30	13,095
Specialized Clinical Research Center Grants (P50)	4	8,534
Research Career Development Awards (K04)	53	9,970
Academic Investigator Awards (K07)	<u>18</u>	<u>19,011</u>
	1,046	85,560

The codes in parenthesis in the above table are the symbols used by NIH to differentiate the various types of grant awards. A description of each of these mechanisms can be found in the Introduction to Volume Three of the publication Vision Research--A National Plan: 1978-1982 (DHEW Publication No. [NIH] 78-1260).

The National Eye Institute complements its research grants with a program of institutional and individual fellowships. The purpose of the program is to equip young investigators with the skills, experiences, and insights necessary for them to embark successfully on a career in vision science, especially its clinical aspects, and other disciplines, such as the basic medical sciences, epidemiology, engineering, and biomathematics.

A total of \$4,593,000 was available for support of vision research training in FY 1980, most of it for the National Research Service Awards (NRSA). The individual NRSA fellowship awards accounted for \$1,778,000, or 39 percent of available training funds. The institutional NRSA training awards accounted for \$2,815,000, or 51 percent of the program. A summary of the training program for FY 1980 follows.

VISION RESEARCH TRAINING FY 1980
(Amounts in Thousands)

	<u>INSTITUTIONAL (NRSA T32)</u>				<u>INDIVIDUAL (NRSA F32)</u>			
	No. of Inst. Awards	Pre- Doctoral	Post- Doctoral	Amount	No. of Ind. Awards	Amount	Total (T & F)	Percent Training Budget
Retinal and Choroidal Diseases	15	10	49	\$ 984*	52	\$ 866	\$1,850	40
Corneal Diseases	10	9	34	750	11	210	960	21
Cataract	3	0	8	98	7	122	220	5
Glaucoma	4	0	15	305	5	95	400	9
Sensory and Motor Disorders of Vision	11	19	33	678	24	485	1,163	25
TOTALS	43	38	139	\$2,815	99	\$1,778	\$4,593	100

* \$13,000 of this total represents NEI's co-funding of two T35s (short-term training program) under the auspices of the NIGMS for four predoctoral positions.

The FY 1980 appropriation for the NEI included \$3,000,000 for research grants for construction of vision research facilities. The program was formally announced on September 19, 1980. Applications will be due in May 1981.

The NEI has made several policy changes of interest to the vision research community. First, the NEI has adopted the NIH guidelines for the New Investigator Research Award (NIRA) program. Basically, this research grant program is intended to support young investigators in the very early stages of their careers. Because of the overall similarities between the NIRA guidelines and the NEI Academic Investigator Award program, we have discontinued use of the Academic Investigator Award mechanism. In addition, the guidelines for NEI Core Grants have been revised. A committee composed of members of the National Advisory Eye Council, a member of the Vision Research Program Committee, and NEI staff met over a six-month period to evaluate all aspects of the Core Grant program. The main concern was the potential for expansion of the Core Grant program at a time when funds for research grants were decreasing. The major policy changes are (1) the maximum amount the NEI will devote to the Core Grant program would be 5 percent of the extramural grant budget, (2) a dollar limit of \$150,000 per year direct costs has been established, (3) the appropriateness of modules within a Core Grant has been greatly clarified, (4) review criteria have been better defined. Both of these policy changes will greatly enhance our ability to implement our research grant programs.

Immunology Workshops

The National Advisory Eye Council, in its 1977 report entitled Vision Research--A National Plan: 1978-1982, identified the need for increased research effort on the immunological aspects of ocular diseases and for recent advances in the general field of immunology to be applied to the study of the visual system. To accomplish this objective, the Council believes that immunologists should be encouraged to become better acquainted with ocular tissue and systems in order that these researchers may exploit the eye as an immunological model.

An attempt to stimulate discussion and collaboration among vision researchers and immunologists was initiated through the development of an immunology workshop series. These workshops were planned by the NEI staff with full assistance from members of the National Institute of Allergy and Infectious Diseases staff in the selection of appropriate conference participants.

The first workshop in this series, "Immunogenetics and Transplantation Immunity," was held on December 5-7, 1979, and identified potentially productive applications of recent advances in immunogenetics and transplantation immunology in eye research.

The following specific recommendations were made by workshop participants:

1. The NEI should develop mechanisms for attracting immunologists to departments of ophthalmology and training them to carry out research on

ocular problems. Veterinary ophthalmologists should be encouraged to work with such immunologists to develop good animal models for basic investigations into ocular immunologic mechanisms.

2. Corneal transplantation studies:

- a. The antigens and cell types in each layer of the cornea which may participate in graft rejection should be defined.
- b. Organ specific antigens in the cornea should be studied.
- c. Investigations should be undertaken on the various mechanisms by which graft rejection can take place.
- d. Graft rejection studies should explore the effect of matching histocompatibility leukocyte antigens (HLA) at both the A and B locus. These antigens appear on the cellular surface of lymphocytes in the peripheral blood and are controlled by genes located at histocompatibility zones A and B on the two number 6 chromosomes in humans.

3. Autoimmunity and infections of the eye: Attempts should be made to define individual antigens responsible for ocular autoimmune diseases such as sympathetic ophthalmia and infectious diseases such as herpetic keratouveitis.

4. True and false malignant lymphoid tumors of the eye: Investigators should determine whether lymphocytes (white blood cells) associated with these tumors are derived from the thymus, fetal liver and gut or lack markers from any of the above tissues; whether the lymphocytes arise from one clone of cells; and what function they perform.

5. Association of HLA antigens with ocular disease:

- a. The extent to which individual immune responses to defined ocular antigens vary should be studied.
- b. Epidemiological studies should investigate suspected associations of certain HLA antigens with ocular diseases.

6. Chemical and physical factors which lead to vascularization of corneal transplants and their resultant rejection should be delineated. Methods should then be sought to limit such vascularization.

7. The mouse should be adopted as a good immunogenetic animal model for study of immunological insult to the eye.

A second workshop entitled "Autoimmune Phenomena and Ocular Disorders" was subsequently convened on March 5-7, 1980, and provided promising avenues for additional research in the immunological mechanisms which govern autoimmunity in relation to specific ocular diseases.

The research priorities developed by the workshop participants are listed below in rank order:

1. Much consideration should be given to the long-term training of both full-time immunologists involved in vision research and ophthalmologists engaged in research on the ocular immune response. A collaboration between these two classes of investigators should be encouraged.
2. Animal models should be recognized and developed, in particular those which represent spontaneous ocular autoimmune diseases. This may be achieved mainly through liaison with the veterinary community. In parallel, experimentally induced autoimmune diseases should be further developed, with the purified ocular antigens.
3. As many specific ocular antigens as possible should be isolated, characterized, and identified from experimental animals and, where feasible, from human subjects.
4. The possible associations between autoimmune ocular diseases in man and histocompatibility (HLA) antigens should be further examined, along with population and family studies. In parallel, the immunogenetics of ocular autoimmune diseases should be further analyzed in experimental animals with well-defined genetic makeup.
5. Longitudinal clinical studies should be conducted on patients identified as being afflicted with various ocular autoimmune diseases. Establishment of the abnormal features in these patients through all phases of their diseases should provide helpful information concerning the respective pathogenic mechanisms.
6. The pathogenic mechanisms involved in autoimmune ocular diseases should be further investigated, both in man and in experimental animals. Better knowledge in this research area should bring about better approaches to therapy.
7. New approaches to immune modulation should be applied to ocular immune diseases such as the use of immunosuppressive agents, suppressor cells, and their products and specific anti-idiotypic immunity.

The third and final workshop in this series, "Infection, Inflammation, and Allergy," was held on June 25-27, 1980, and represented the completion of the NEI initiatives in the field of immunology for FY 1980. This workshop provided a forum for discussion of potentially promising approaches to minimizing the destructive effects of inflammation associated with ocular disease while maximizing the protective effects of the inflammatory process.

The following specific recommendations were formulated by workshop participants:

1. Early biochemical events in ocular inflammatory disease(s) requires investigation. Chemical mediators need to be identified which can regulate chemotaxis of inflammatory cells and function as causal agents

for neovascularization in both the cornea and the retina. The events in the inflammatory response should be differentiated so that components deleterious to the host may be avoided.

2. Development of naturally occurring and/or genetically defined animal models of human ocular infections, inflammatory and allergic diseases should be encouraged. Increased recognition and promotion of the use of such animals should be fostered.
3. Support should be provided for investigations of the primary defense mechanisms exhibited by ocular mucous surfaces to elucidate which cellular events in barrier disruption lead to inflammation and ultimate structural damage.
4. The effects of specialized nonlymphoid inflammatory cells including mast cells and eosinophils should be determined in human and animal model systems of inflammation.
5. Studies should be continued on the evaluation of the individual types of interferon alone, in mixtures, and in combination with antiviral drugs on such ocular diseases as recurrent stromal herpes keratitis, herpes zoster, and adenovirus infections.
6. The potential use of anti-idiotopic antibodies either directly or through the subsequent generation of appropriate suppressor cells for therapy (and possible prevention) of ocular infections, ocular tumors, and uveitis should be explored. These specific stimulators should also be utilized in development of diagnostic techniques for specific ocular inflammatory diseases.
7. The development of knowledge about the chemical nature of glucocorticoid receptors and the modification of such receptors through drug action should be pursued in ocular tissues.
8. An increased knowledge of nonsteroid drugs which are capable of inhibiting individual rate limiting steps in the inflammatory process is needed.
9. Drug delivery systems which either increase the penetration or permeability of drugs through topical application to the eye or through subconjunctival implantation are in urgent need of development.
10. New antiviral drugs should be developed, especially those compounds which can eradicate herpes virus at the ganglion level.
11. Evaluation of new antibiotics and antifungals for treatment of ocular disease should be carried out both as to increased ocular penetration and pharmacokinetics as well as toxicity of such compounds.

Upon completion of the revision and editing of the individual manuscripts in each workshop, the proceeding of that workshop will subsequently be published as a special supplement to the journal Immunology Abstracts in 1980 and 1981. Copies of the three-volume set of publications will be provided to each of the workshop participants and to interested persons in the vision and immunology communities through appropriate journal announcements.

RETINAL AND CHOROIDAL DISEASES

Introduction

Retinal and choroidal diseases are the leading causes of blindness in the United States. These diseases interfere with communication, seriously restrict employment opportunities and limit mobility for a large segment of our population. Further evidence of the devastating effects of retinal diseases is that ophthalmologists consider them to be the most difficult ocular disorders to treat, and the most in need of basic as well as clinical research.

Diseases of the retina are diverse in origin and the research programs to attack them are complex. In previous years, the subprogram's activities, as outlined in Vision Research - A National Plan: 1978-1982, were divided predominantly into fundamental and clinical studies. A more appropriate division which demonstrates the interrelationship between basic and applied research is under consideration by the Retina Program Planning Panelists. As proposed, the retina program would be divided into four comprehensive sub-programs to include:

- A. Vascular, Neoplastic and Inflammatory Disorders of the Retina and Choroid
 - o Diabetic Retinopathy, Other Vascular and Circulatory Disorders and Retinopathies
 - o Inflammatory Disorders
 - o Tumors
- B. Degenerative Disorders of the Retina
 - o Developmental and Hereditary Disorders
 - o Macular Degeneration
 - o Retinal Detachment, Vitreous Disorders and Trauma
- C. Fundamental Processes and Retinal Disorders
 - o Photoreceptors, Visual Pigments, and Transduction
 - o Interaction between Visual Cells and Pigment Epithelium
 - o Retinal Organization, Neurotransmission, and Visual Function
 - o Non-Invasive Techniques in the Study of Retinal Disorders
- D. Special Areas of Future Interest
 - o Toxic, Nutritional and Environmental Disorders
 - o Low Vision and Myopia
 - o Retinal Regeneration and Glial Cells and the Retinal Environment
 - o Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models

The NEI's Retinal and Choroidal Diseases Program supports a cadre of research scientists from the basic and clinical areas such as biochemistry, biomedical engineering, cell biology, electrophysiology, epidemiology, molecular biology, genetics, immunology, molecular biology, neuropharmacology, ophthalmology, optometry, pathology and psychophysics. They are all striving to prevent, diagnose, and understand the mechanisms of visual disorders, and to improve the quality of life through treatment of these diseases.

In FY 1980, the Extramural and Collaborative Program's Retinal and Choroidal Diseases Branch has been responsive to the objectives outlined in Vision Research - A National Plan: 1978-1982. Although significant advances have been made in most subprograms, the staff has decided to highlight, in the annual report, progress in the following areas: diabetic retinopathy, allergic uveitis, hereditary and other retinal degenerations, ocular tumors, photoreceptor renewal, and visual transduction. Also included in this are the recommendations of the Ocular Melanoma Task Force which convened April 17-18, 1980 at the National Institutes of Health to evaluate recent literature on ocular melanoma and to recommend research initiatives aimed at the improved diagnosis and treatment of this disease.

Diabetic Retinopathy

Diabetic retinopathy, besides causing severe visual impairment, is one of the major leading causes of new cases of blindness in the United States. It is characterized pathologically by excessive leakage of fluid from retinal capillaries, microaneurysms, occlusion of retinal vessels, and proliferation of newly-formed blood vessels and fibrous tissue along the inner surface of the retina. Impaired vision results from:

- o Leakage from capillaries into the macula causing swelling and distortion with impaired reading vision, sometimes progressing to legal blindness.
- o Impairment of retinal blood supply leading to loss of part of the visual field, including reading vision when the macula is involved.
- o Bleeding from the newly formed blood vessels into the vitreous and detachment or distortion of the retina caused by contraction of the fibrous tissue and resulting in total or nearly total blindness.

The NEI has utilized a comprehensive approach to reduce or eliminate the ocular complications from diabetes which involves testing the safety and efficacy of various therapies, determining risk and protective factors, and understanding how the observed pathological changes result in visual disorders.

In 1973, 46 percent of ophthalmologists surveyed considered retinal research to be the most inadequately developed, especially research on hereditary retinal and macular degenerations and diabetic retinopathy.¹ In just seven years, intensive research in the area of diabetic retinopathy has made the latter part of the previous statement obsolete. The use of randomized controlled clinical trial methodology to determine the safety and efficacy of photocoagulation in treating diabetic retinopathy is without doubt one of the major accomplishments in ophthalmic research of this decade.

The Diabetic Retinopathy Study (DRS) showed that photocoagulation treatment can reduce the risk of severe visual loss by 60 percent in people with moderate to severe diabetic retinopathy. It is estimated that 300-500 thousand known diabetics have retinopathy serious enough to warrant consideration for this treatment. Photocoagulation is now offered at many hospitals and most university medical centers throughout the United States. The safety and efficacy of this technique for use by practicing ophthalmologists is assured due to the identification of four risk factors which indicate when a patient is at high risk of developing severe visual loss.² Thus, this information can be used to determine when to photocoagulate eyes of patients with diabetic retinopathy.

Since the above mentioned studies were performed in people with moderate to severe diabetic retinopathy, some clinical researchers have postulated that earlier treatment of diabetic retinopathy by photocoagulation may slow or even prevent visual loss. Thus, a new multicenter trial, the Early Treatment Diabetic Retinopathy Study was begun in 1977 and if this study is as successful as the DRS, it may be possible to enhance the quality of life for a greater number of people with diabetes by maintaining their visual function.

Restoring sight or decreasing the progression of visual impairment through the application of photocoagulation could not have been achieved without studying a large, well-defined population of patients whose ophthalmologists were convinced that the only ethically and scientifically feasible way to determine efficacy of this treatment was through a randomized controlled clinical trial. The ultimate success of photocoagulation therapy will depend on ophthalmologists and patients with diabetic retinopathy being aware of its usefulness and the safe application of this technique by all ophthalmologists.

The potential impact of the DRS on the lives of many who would have otherwise been visually impaired or blind for a significant portion of their adult lives points to the importance of further clinical research on diabetic retinopathy and related diseases. Other randomized controlled clinical trials which currently receive NEI grant support are:

Branch Vein Occlusion Study. The objective of this study is to determine whether argon laser photocoagulation can (1) prevent the development of neovascularization, (2) prevent vitreous hemorrhage in branch vein occlusion patients with neovascularization and (3) improve or maintain visual acuity in patients who have macular edema secondary to branch vein occlusion.

Macular Photocoagulation Study. The objective of this study is to determine whether the use of argon laser photocoagulation to obliterate extrafoveal choroidal neovascularization is of value in preventing permanent loss of central visual function in patients with senile macular degeneration and in patients with presumed ocular histoplasmosis.

Photocoagulation in Disciform Macular Disease. The objective of this study is to assess the therapeutic value of argon laser photocoagulation in the disciform process of senile macular degeneration.

While research on the treatment of diabetic retinopathy will continue in the 1980s more attention will be directed toward prevention, early diagnosis, and understanding the underlying mechanisms in this disorder.

Epidemiological studies are extremely important in preventive medicine because they can help determine the magnitude of the problem, provide a profile of the susceptible person, and determine what risk or protective factors are associated with the disorder. Klein at the University of Wisconsin³ analyzed the medical records of over 5,000 diabetic individuals from southern Wisconsin. He recorded a bimodal distribution for age of onset of diabetes with peak frequencies in the 10-14 year age band and the 60-64 year age band; diabetes was diagnosed in approximately fifteen percent of the population before the age of twenty-five years. These results confirm previous studies on the prevalence of diabetes.

At the University of Minnesota, Ramsey and his colleagues are testing the hypothesis that susceptibility to microangiopathy in diabetics is genetically determined. The group is trying to determine whether one or more "microangiopathy genes" are linked to histocompatibility (HLA) genes. It has been well substantiated that diabetic patients with microangiopathy are more often positive for HLA B8, B18 and B8/15 and that normal volunteers are more often positive for HLA B7 and B12. When the HLA antigens from juvenile insulin dependent diabetics (IDDM) with non-proliferative diabetic retinopathy are compared with the HLA antigens from diabetics with proliferative diabetic retinopathy (PDR), patients with PDR are less often positive for HLA B7 and significantly more often positive for HLA B15. These observations suggest that HLA B15 may be a susceptibility marker for PDR.⁴

Since several autoimmune disorders in humans have been associated with HLA B8 and there is evidence for abnormal immune mechanisms in some cases of IDDM, it is important to identify the allele primarily associated with diabetes mellitus in humans. Ramsey and his colleagues studied the interrelation between IDDM and the HLA B8 and the D locus in forty one unrelated patients. They demonstrated significant increases in the frequencies of HLA B8, B18 and Dw3 and significant decrements in the frequencies of HLA B7, B12 and Dw2 in patients with IDDM. The investigators concluded that the HLA B8 excess seen in diabetics was secondary to the excess of HLA Dw3, thus indicating that the pathogenesis of the disease is closely associated with the D locus of the major histocompatibility system.⁵

Bresnick at the University of Wisconsin is employing a non-invasive electrophysiologic technique to identify eyes that are at high risk of developing PDR.⁶ Preliminary data from diabetic patients with retinopathy ranging from mild non-proliferative to proliferative showed a reduction in ERG oscillatory potentials with advancing retinopathy. This recently initiated study also has the potential for helping identify candidates for vitreous surgery from among diabetic patients with vitreous hemorrhage. For the surgeon, evaluating patients with cloudy media by existing methods is difficult, and such a diagnostic technique would be extremely useful.

The blood-retinal barriers (BRB) appear to be located at the endothelium of the retinal capillary and at the retinal pigment epithelium; in diabetic retinopathy the BRB is disrupted. Neither the basic mechanism of disruption nor the factors responsible for its repair have been determined. However, researchers supported by NEI are actively seeking answers to this problem.

One approach is to compare the morphology of normal retinal vessels with that of abnormal retinal vessels. Frank at Wayne State University is using⁷ the scanning electron microscope to study small retinal blood vessels. Initial observations of retinal vessels from a patient with proliferative retinopathy revealed a bulbous crenulated microaneurysm with several attached acellular capillaries whose basement membrane showed multiple ridges and furrows, rather than a smooth surface. This technique will be useful in determining the architecture of retinal blood vessels and in correlating morphology with biochemical and physiological changes in microangiopathy.

Wallow at the University of Wisconsin is examining the morphology of the BRB by electron microscopy.⁸ He found that in the new vessels, the blood-retinal barrier was altered by the formation of fenestrae bridged by diaphragms. Occasionally, tight junctions between endothelial cells appeared widened. Thus, the characteristic "leakiness" observed clinically by fluorescein angiography may be caused by fenestrae and incompetent tight junctions.

Some evidence is emerging from the works of Peczon and Mukai⁹ in Boston that the alterations in basement membranes in diabetic retinal vessels may include not only a thickening of the basement membrane but that the cellular metabolism may also be different from the retinal vessels of non-diabetics. Autoradiographic studies of the basement membrane of normal and streptozotocin-induced diabetic rats showed that the basement membrane had increased perivascular collagen fibrils.

How the BRB in diabetic eyes recovers after chronic photocoagulation was examined by Wallow and Davis.¹⁰ In donor eyes from diabetics treated with photocoagulation, they found that burns of 0.5 seconds or longer resulted in complete destruction of the neurosensory retina with only the retinal pigment epithelium providing the resource of repair to bridge the retinal defect created within the center of the burn; the pigment epithelium cells were often devoid of pigment. Following less severe burns from argon photocoagulation of 0.1 second or less, retinal glial cells participated in the reparative process

together with the pigment epithelium cells and the pigment-laden macrophages. Such information is extremely important since it can be utilized to correlate safe levels of laser energy with efficacy of treatment.

Scientists are just beginning to probe into the mechanisms responsible for description of the blood-retinal barrier in diabetic patients. A thorough analysis of the problem requires expertise in such disciplines as cell biology, morphology, physiology, biochemistry and endocrinology. To facilitate the process, the RCDB is planning to convene a multi-disciplinary task force in the Spring 1981 to discuss research needs in the area of diabetic retinopathy. This activity will complement the NEI's third program planning exercise currently underway.

Hereditary and Other Retinal Degenerations

Hereditary and developmental disorders are responsible for an estimated thirty-three percent of all blindness among school children in the United States. Presently, little can be done to prevent the onset and progression of most of these disorders, however, several researchers within the past year have made significant contributions in early detection of the disorder, identification of genetic carriers and evaluation of nutritional therapies.

Retinitis Pigmentosa. Light-evoked electrical responses from the human eye recorded at the cornea have provided criteria for evaluating the functional integrity of specific layers of the human retina. The a-wave and b-wave depend on both rod and cone systems. The c-wave depends on the rod system and a healthy pigment epithelium. Thus, electrophysiological tests can be utilized to determine cell layer interaction and normalcy of cells.

Approximately 35 percent of patients with retinitis pigmentosa (RP) are males with no family history of RP, or males with one or more affected male relatives and no affected female relatives. Berson and his colleagues at Harvard University¹¹ were able to show that the full field electroretinograms (ERGs) of female carriers were either reduced in amplitude to white light under dark adapted conditions or delayed in cone b-wave implicit time, or both. Daughters of carriers had either normal ERGs or abnormal ERGs similar to those recorded for carriers. Thus, ERG testing of female relatives of males with retinitis pigmentosa can establish for a given family whether the mode of inheritance is X-chromosome linked or autosomal recessive.

Preliminary data from Corwin's laboratory at the University of Rochester suggest that some psychophysical tests can be used to discriminate between normal volunteers and patients with RP.¹² A procedure for measuring two-flash resolution was developed and implemented using a microcomputer-controlled interactive tracking procedure. The mean threshold inter-flash interval for a normal population was 60.0 msec compared with 79.2 msec for one confirmed RP patient. This test yielded reliable results when administered to inexperienced subjects and, it may have potential use for large-scale visual screening of a population at risk.

Berson and his group are attempting to identify abnormal structures in the retinal cell layers of RP patients.¹³ The postmortem histological analysis of an eye from a patient with a slowly progressing form of RP (visual acuity of 20/300) revealed a reduced number of cones with shortened outer segments in the fovea and some rods on the fovea slope, the absence of photoreceptors in the mid periphery, and preserved rods and cones anterior to the zone of bone spicule pigmentation. The pigment epithelium cells adjacent to the photoreceptors in the central retina contained decreased melanin, and autophagic vacuoles in the cone photoreceptors.

It has been shown that patients with some types of mucopolysaccharidoses have RP. The levels and kinds of glycosaminoglycans (GAGs) were measured in a pigment epithelium cell line derived from a patient with X-chromosome-linked RP.¹⁴ The GAGs secreted by these cells were similar to that secreted by normal cell lines. Thus, a defect in GAG metabolism appears not to be associated with X-chromosome-linked RP.

Gyrate Atrophy. Gyrate atrophy of the choroid and retina (GA) is an inherited progressive chorioretinal degeneration. The visual abnormalities in GA are characterized by the onset of myopia and decreasing peripheral and night vision in late childhood or early adolescence. Progressive destruction of the visual fields results in tunnel vision and eventual blindness by the age of fifty. Metabolic studies have shown that GA patients have a deficiency in the enzyme, ornithine-amino-transferase (OAT). Since this is an inheritable disease, researchers have been developing sensitive and reliable assays for detecting GA and determining whether a particular form of GA can respond to therapy. O'Donnell at the University of California in San Francisco¹⁵ and Valle at Johns Hopkins University¹⁶ have demonstrated that cultured cells from GA patients have decreased levels of OAT. In addition, the former investigator was able to assay cells obtained by amniocentesis for OAT activity. Thus, this assay can potentially be used in genetic counseling.

Detecting the different mutations leading to OAT deficiency is essential in understanding variability among the clinical manifestations of the disease. Valle and his colleagues¹⁶ have developed a genetic complementation assay to test for different mutations which involves hybridizing fibroblasts from different patients with GA and measuring the recovery of OAT activity. Fourteen cell lines have been tested so far and none were shown to be complementary. Thus, all tested cell lines appeared to have allelic mutant genes.

Valle and Kaiser of the National Eye Institute's Clinical Branch have treated a small number of GA patients by diet modification. Eight patients have been on arginine-deficient diets from 3-28 months and have had a 2-6 fold reduction in plasma ornithine.¹⁶ One patient who had been on the diet for over 24 months has shown improvement in dark adaptation, ERG and visual fields. In another GA patient who is vitamin B₆-responsive, daily doses of vitamin B₆ were effective in lowering serum ornithine levels with a concomitant improvement in ERG response.¹⁷

Animal Models. Animal models which mimic human disorders are valuable resources to the investigator. Three models of hereditary retinal degenerations have been studied extensively: the dog model (specifically Irish setters, Norwegian elkhound and miniature poodle), the Royal College of Surgeon (RCS) dystrophic rats, and several mutant mouse genotypes.

A mutant mouse, pcd/pcd, in which photoreceptor cells degenerate slowly over the course of a year is being studied by La Vail at the University of California, San Francisco.¹⁸ Rods were found to degenerate faster than cones. In the two month old mutant mouse, the rate of rod outer segment disc synthesis and the disc shedding values after light onset were approximately one-half that of the values obtained in control mice.

Using the dog model, Aguirre at the University of Pennsylvania¹⁹ is studying the kinetics of rod outer segment renewal in Irish setters with rod-cone dysplasia. He has demonstrated a lack of synchronous production of new rod outer segment discs at the time normal photoreceptors are elongating. In addition, no defect in phagocytosis by the pigment epithelium was detected.

The abnormally high levels of cGMP in the retinas of dogs with rod-cone dysplasia results from the lack of photoreceptor-specific phosphodiesterase (cGMP-PDE). The activity of this enzyme can be regulated by calmodulin, a calcium-dependent protein activator. In examining the relationship between calmodulin and cGMP metabolism during photoreceptor differentiation, Aguirre and his colleagues found that in the normal retina, cGMP-PDE changes from calmodulin-dependent to calmodulin-independent, whereas in affected dogs, cGMP-PDE fails to become calmodulin-independent with aging.²⁰ These observations suggest that the disease may be modulated or corrected by delivering calmodulin to the visual cells.

Ultrastructural and biochemical studies have been performed on the retinas of RCS rats to define the cellular and molecular events of retinal degeneration. La Vail,¹⁸ demonstrated that changes occur in the distribution and quantity of mucopolysaccharides of the interphotoreceptor matrix in RCS rats before cell death; such changes may be involved in the mechanism of photoreceptor cell death in these animals. A decline in opsin synthesis²¹ was the only detectable change in protein synthesis observed in RCS retinas.

Renewal Processes in Photoreceptor Outer Segments

The shedding of photoreceptor outer segments and subsequent phagocytosis by the retinal pigment epithelium have in recent years been established as normal events in the vertebrate retina. A reasonable hypothesis is that disturbances in the delicate balance between outer segment degradation and reassembly may underlie several forms of retinal degenerative disease. Investigators have increasingly directed their research towards better understanding those processes which maintain or disrupt this balance. NEI intramural scientists and grantees have been the leading investigators in this promising area of research.

The classic studies of Young^{22,23} established that rod photoreceptor outer segment membranes are not static structures, but structures which are continually renewed. Despite structural differences between rod and cone outer segments, it is now clear that renewal is a universal feature of vertebrate photoreceptors.^{24,25}

La Vail²⁶ discovered that rod outer segment shedding was not a continuous, random process, but was a rhythmic event synchronized by the daily light cycle. This unexpected finding galvanized new research efforts into all aspects of this phenomenon. Studies by Young,²⁷ Bunt,²⁸ and Anderson and Fisher²⁹ later demonstrated the cyclic pattern of shedding in cone outer segments. The timing of outer segment membrane shedding by rods and cones, however, is 12 hours out of phase. Rod shedding occurs near the beginning of the light cycle; cone shedding occurs at the beginning of the dark cycle. Regular cycles of disc shedding continue in rats for at least 12 days in constant darkness.³⁰ Thus, the light/dark cycle in mammals may entrain an endogenous circadian rhythm which more directly controls the shedding process.^{31,32,33} Constant light dramatically reduces rod outer segment disc shedding indicating that a dark-dependent control process is operative as well.

The circadian nature of the outer segment shedding process has prompted a search for extraocular control mechanisms. The pineal gland was implicated in early experiments by La Vail^{30,38} who discovered that reserpine (a drug known to block other circadian rhythms by depleting pineal norepinephrine) abolished shedding. However, shedding was quickly shown to continue after surgical removal of the pineal gland, the superior cervical ganglion,³⁵ the pituitary and parathyroid gland and after the cutting of the optic nerves.³⁶⁻³⁷ In both frogs and rats it has been demonstrated in eye-patching experiments that shedding may be initiated monocularly.^{38,39} Finally, in perhaps the definitive experiment, Besharse et al.⁴⁰ have established culture conditions for open frog eye cups in which it is possible to demonstrate light-evoked disc shedding. Taken together, these observations clearly indicate that the major controlling processes of the shedding phenomenon are intrinsic to the eye.

This discussion has focussed on the degradative aspects of the photoreceptor renewal process. But, it is now evident that photoreceptor membrane assembly, and RNA,^{31,33,41,42} protein, and glycoprotein synthesis are also influenced by the photoperiod. Photoreceptor renewal emerges from these studies as the end product of a series of complex, highly-orchestrated metabolic processes. Promising new approaches to the study of retinal degenerative diseases have been made possible.

Visual Transduction in Vertebrate Rod Photoreceptors

A central problem in visual photoreceptor physiology, and in the physiology of all excitable tissues, remains unanswered: What mechanisms bring about the generation, amplification, and modulation of transmembrane ionic currents? Specifically, what process links the absorption of light by rhodopsin molecules in the rod disc membrane with the conductance mechanism of the physically separate plasma membrane? Recent research, much of it

conducted by NEI grantees, has led to important findings which bear on this problem.

Two salient points are generally agreed upon. A transmitter (unknown) must link the site of photon absorption and the plasma membrane. Secondly, many sodium channels are inactivated by a single photon absorption, that is, an amplification process takes place.⁴³

Hubbell and Bownds⁴⁴ have recently reviewed the two general schemes which researchers have used to account for these phenomena. "The calcium hypothesis"⁴⁵ and similar models depend upon compartmentalization of the transmitter by the disc membrane and the participation of rhodopsin in transmembrane processes. Alternatively, enzymatic schemes have been proposed which do not depend on transmembrane phenomena and involve only events at the membrane surface.

The calcium hypothesis holds that calcium accumulates within the discs in the dark-adapted state and is released into the cytoplasm upon rhodopsin activation. The released calcium is translocated in such a way as to inhibit sodium conductance.

Indirect evidence in favor of the calcium hypothesis is strong. Fung and Hubbell⁴⁶ by comparing the lactoperoxidase catalyzed iodination and papain proteolysis of rhodopsin in native and reconstituted disc membranes have unequivocally demonstrated the transmembrane orientation of rhodopsin. Hargrave and his associates have provided other evidence^{47,48} which relates to the transmembrane organization of the rhodopsin molecule. The results of photochemical labeling experiments with membrane impermeable probes indicate that the rhodopsin carboxy-terminal region is located at the cytoplasmic (external) surface of the disc membrane while the amino terminus is located at the intradiscal membrane surface.⁴⁹ Thus, rhodopsin is physically situated within the disc membrane in such a way that gated calcium channel functions or other transmembrane functions may be mediated.

While several experiments have indicated that calcium participates in the excitatory process, direct evidence of an amplified release of calcium is lacking. Brown and coworkers⁴⁹ have shown that an intracellular injection of calcium hyperpolarizes the photoreceptor, mimicking the effects of light. Several groups have found that calcium chelating agents reduce photoresponses of rod receptors.^{49,50} Many investigators have demonstrated a light activated release of calcium from native and reconstituted disc membranes. The estimated amounts of calcium released, however, do not exceed 1.0 calcium ion per activated rhodopsin molecule. Amplification of the calcium response in these systems has yet to be demonstrated, and may in vivo, depend on intact rod structure. An alternative explanation holds that calcium release sets in motion a cascade of events which result in the amplified attenuation of sodium conductance.⁵¹

The second type of scheme which has been proposed to account for transmission and amplification involves enzymatic processes. Here cyclic 3', 5' guanosine monophosphate (cGMP), present in unusually high levels in photoreceptor outer segments, has been proposed to play a central role in transduction. A number of investigators have shown that light brings about a dramatic reduction in the intracellular cGMP concentration and that the reduction is largely mediated by phosphodiesterase. Woodruff et al. have established a number of correlations between cGMP levels and permeability changes in rod outer segments.^{52,53} Bleaching of a single rhodopsin molecule was found to bring about the disappearance of 10^4 - 10^5 molecules of cGMP. The half-time of the decrease in cGMP was about 125 milliseconds (msec.) and the latency was less than 50 msec. A steady-state level of cGMP was reached within seconds of illumination and varied as a function of the light intensity. Changes in cGMP levels and the suppression of plasma membrane permeability occurred over the same range of light intensity, and were similarly affected by pharmacological agents.

Changes in cGMP levels occur rapidly enough and with sufficient amplification to be involved in the rod phototransduction process. Miller and Nicol⁵⁴ have recently reported, in support of this possibility, that intracellular injection of cGMP increased both the latency and amplitude of the rod light response. It is also entirely possible that cGMP may subserve some other function, possibly visual adaptation.

Whether cGMP is the transmitter which links photon absorption with permeability changes of the rod plasma membrane or whether, as now seems likely, other transmitters (e.g., calcium) or steps are involved remains to be proven. Yau, Lamb, and Baylor⁵⁵ have introduced a novel "suction" electrode technique, making possible the study of the photoresponses of single photoreceptor outer segments. It should soon be possible to compare the biochemical measurements here reviewed with rod outer segment conductance measurements--an exciting experimental opportunity for investigators in the field.

Allergic Uveitis

The importance of immunology in ocular diseases has been emphasized by NEI in its sponsorship of three immunology workshops this fiscal year. The purpose of these workshops was to bring together eminent immunologists and ophthalmologists to discuss the state-of-the-art in immunology. NEI grantees within RCDB participated in these very important workshops. Currently, the NEI supports eighteen grants in its Retinal and Choroidal Diseases program related to the immunology of the eye for a total of \$1.1 million dollars.

Allergic uveitis is a non-infectious inflammatory disease of the uvea and is thought to arise from an immunologic insult. The principal causes of blindness from inflammatory diseases of the retina and the choroid are destruction of the photoreceptors in the macula, damage to the nerve fibers that transmit visual impulses to the optic nerve, and opacification of the vitreous overlying the inflammatory lesion.

The etiology of allergic uveitis is still unknown. However, an immune response to ocular antigen has been detected in some patients with uveitis. Wacker and his co-investigators at the University of Louisville in Kentucky, along with Nussenblatt, Gery and Ballintine from the NEI's intramural program, have shown that a soluble retinal antigen (S-antigen) purified from human and bovine retinas can stimulate the lymphocytes of some patients to divide.⁵⁶ This phenomenon was observed in patients who had both active and inactive retinal lesions. Some patients with posterior uveitis responded to crude retinal extracts but not to the purified S-antigen, indicating that other retinal antigens may also be involved in posterior uveitis. Neither control subjects nor patients with anterior uveitis manifested a positive response to the S-antigen. These responses may play some role in the pathogenesis of the disease. The question of whether the immune response is the cause of or the result of inflammation remains to be answered.

Other researchers are using animal models of experimental allergic uveitis (EAU) to investigate the possible immunologic etiology of allergic uveitis. Three animal models for posterior uveitis are presently being studied: (1) a rhodopsin-induced guinea pig model, (2) an S-antigen-induced EAU rabbit, and (3) guinea pig models. Wacker was able to show that the dose of S-antigen used to induce uveitis in the rabbit was associated with the severity of the pathologic response.⁵⁷ High doses of S-antigen (50 micrograms) produced an acute and severe inflammation including fibrinoid necrosis of the retinal vessels whereas low doses (10 micrograms) produced a less severe inflammation with mononuclear infiltrates. Meyers-Elliott at the University of California, Los Angeles, has induced EAU in guinea pigs by injecting them with 500 micrograms of purified bovine rhodopsin in complete Freund's adjuvant.⁵⁸ The retinal pathology of EAU was characterized by destruction of the photoreceptor cell layer. At no time was a substantial cellular infiltrate noted in the retina. Wacker and his co-workers showed that guinea pigs sensitized with disc membranes developed clinical symptoms of EAU between 3 and 6 weeks.⁵⁹ Histologically, these animals exhibited a mild non-granulomatous choroiditis with little or no involvement of the anterior uvea segments.

Silverstein and co-workers at Johns Hopkins University are investigating the immune response in inflammatory ocular disorders.⁶⁰ They have recently described an intraocular immune response accompanying a lymphokine-induced [Sea Star Factor, (SSF)] inflammatory response in rabbits.⁶¹ The SSF produced earlier and more devastating changes in the retina and the uveal tract than the control antigen, ovalbumin. The time of appearance of these abnormal changes suggest that SSF produced far more inflammation than could be due simply to its immunogenic properties alone. A series of lethally x-irradiated rabbits developed neither retinitis nor vitritis during the early post-inoculation period. Thus, the damage seen by SSF may be attributed to its ability to attract and to activate macrophages with resultant destruction of ocular tissue.

Tumors

Ocular tumors are the only eye disorder which can result in death. Tumors of the eye arise principally in the retina and the uvea, although some originate from other areas such as the lids, conjunctiva, and orbit.

Retinoblastoma is the most common ocular tumor among children; it rivals neuroblastoma as the most common congenital tumor. According to various studies in the United States and in Western Europe, retinoblastoma occurs at the rate of one per 23,000 live births. The most alarming feature of retinoblastoma is that it appears to be increasing in frequency. Although these tumors can be treated with up to ninety percent effectiveness by enucleation, blindness and a reduced quality of life are inescapable.

There is some evidence that hereditary fragile chromosomal regions (the long arm of chromosome 13) may be related to the development of retinoblastoma. Murphree at Children's Hospital in Los Angeles is attempting to define the linkage between a marker enzyme, esterase D chromosome 13, and the hereditary forms of retinoblastoma.⁶² The clinical application of linkage analyses can be used for genetic counseling and for early identification of persons who are at risk of developing the disease.

Albert and his colleagues at the Massachusetts Eye and Ear Infirmary in Boston have demonstrated that fibroblasts from patients with hereditary retinoblastoma are more sensitive to killing by x-ray than those from patients with sporadic retinoblastoma or normal volunteers.⁶³ These studies offer preliminary evidence that x-ray sensitivity may be related to a deletion observed in a specific region of chromosome 13.

The role of the immune system in preventing tumors or containing their growth is poorly understood. Albert is studying a lymphocyte-mediated cytotoxic response to human retinoblastoma cells.⁶⁴ The assay system involves the use of human retinoblastoma derived cells (cell line Y-79) in a 14-hour radioactive chromium release assay. Lymphocytes from healthy donors showed greater cytotoxic activity against the Y-79 cells than did lymphocytes from patients with retinoblastoma; these results would suggest some type of immune surveillance in healthy donors.

The treatment of ocular tumors is an area of interest to several vision researchers because of the tumor's potential to cause vision loss and death if metastasis occurs. The application of drug delivery systems for chemotherapy is being explored by Liu in Boston.⁶⁵ He is experimenting with a silicone balloon injected with 1,3-bis (2-chloroethyl)-1 nitrosourea (BCNU) and implanted in the episclera of rabbits who have developed Greene's malignant melanoma. In ten eyes so treated, tumor growth was delayed, and eight of the ten eyes retained normal size and shape thirty days after treatment. Sery at Wills Eye Hospital in Philadelphia has employed a novel form of therapy, photodynamic inactivation, to treat rabbits whose eyes have successful implants of retinoblastoma or Greene's malignant melanoma.⁶⁶ This approach

is based on the theory that in the presence of visible light, some porphyrins release molecular oxygen causing photooxidative damage to a biological system. Since a variety of neoplasms preferentially take up hematoporphyrin and hematoporphyrin derivatives, the feasibility of such a technique being useful appears good. Data exist in the literature which support the effectiveness of photoinactivation in treating tumors in both animals and humans. Using the rabbit model, Sery had shown that tumor death occurs in animals sensitized with hematoporphyrin for three days prior to irradiation with a cold red beam at 620-640 nm from a dye laser. He and his clinical associates have received approval from the Food and Drug Administration to apply this therapeutic procedure to human tumors.

Malignant melanomas of the uvea are the most frequent type of ocular tumor and comprise eighty percent of ocular malignancies in adults. Differential diagnosis is difficult and the appropriate management of these tumors is controversial. In addition, little is known about the epidemiology, etiology, natural history or biology of the tumor. One of the major activities of the RCDB this year was to assemble a task force to recommend research initiatives in this area following evaluation of the literature. A summary of its recommendations follows.

Epidemiology of Ocular Tumors.

- o Conduct demographic studies to examine risk factors, such as socioeconomic group, occupation, geographic locale, latitude, eye, skin and hair color, exposure to ultraviolet light, and presence of cutaneous melanomas.
- o Collect complete family history data.
- o Use appropriate control groups for data analysis.

Biology of Ocular Tumors.

- o Investigate the etiology of ocular tumors, including the role of environment, chemical, genetic and viruses.
- o Establish ocular melanomas cell lines, study the growth properties of tumor cells in culture, and investigate factors which influence the growth rate of tumor cells.
- o Determine the oncogenic potential of various cell types and lesions.
- o Characterize tumor specific antigens and determine markers of oncogenity.

Diagnosis of Ocular Melanomas.

- o Develop improved non-invasive methods for sizing tumors and determining growth rate in vivo.
- o Develop non-invasive methods to differentiate in vivo between malignant melanomas and nevi.

Pathology of Ocular Melanomas.

- o Standardize clinical and pathological terms.
- o Standardize terminology for classifying lesions.
- o Define methods to predict the prognosis from tumor pathology such as staging of tumors.
- o Determine which cell types are associated with malignancy.

Natural History Studies.

Set up regional centers to collect data on patients with melanomas using a standardized form. The type of data collected would include:

- o Time when tumor was initially diagnosed.
- o Tumor size.
- o Growth rate of tumor.
- o Description and location of lesion.
- o Survey of all cutaneous surfaces for the presence of melanomas.
- o Complete metastatic work-up including spleen and liver scans, liver function tests, general physical examination and chest x-ray.
- o Type of intervention, if any.

Prospective Studies. The task force suggested that a randomized controlled clinical trial be conducted using as the study group ocular melanoma patients who present with extrascleral extensions. The suggested type of intervention was surgery with newer forms of adjunctive therapy such as thymosin, interferon or transfer factors.

Chemotherapy and other forms of immunotherapy such as BCG were not highly recommended. The protocol for such a trial should include a minimum of treatment modalities and should be written in cooperation with the participating research clinicians. In addition, a determination should be made with respect to the sample size required for a meaningful study and the number of ocular melanoma patients eligible for the study.

Underlying the recommendations was the task force's belief in a need to share resources, a need for greater cooperation among ophthalmologists, and a need for increased communication among ophthalmologists, epidemiologists, and medical oncologists.

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Retinal and Choroidal Diseases

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CORNEAL DISEASES

Introduction

The Corneal Diseases program of the National Eye Institute provides the focus within the federal government for research support for the investigation of disorders of the cornea, lids, conjunctiva, the lacrimal gland, diseases of the orbit and external eye, and refractive errors and injuries of the cornea. Although lesions of the cornea account for only approximately 6 percent of all cases of blindness in the United States, diseases and disorders which affect this tissue constitute 62 percent of the total incidence of acute and chronic disorders, diseases and injuries which affect the eyes. Ocular infections, conjunctival allergies and traumatic and/or foreign body injuries to the cornea constitute the bulk of these eye problems.

In this report, only a few of the research areas of greatest programatic activity will be considered in relation to the research priorities for the Corneal Diseases program as outlined in the 1977 report of the National Advisory Eye Council, Vision Research--A National Plan: 1978-1982. These areas are: ocular herpetic infections, trophic influences on corneal epithelium, control of corneal hydration and transparency and collagen type distribution in the cornea.

Ocular Herpetic Infections

Viral keratitis and other keratopathies represent debilitating diseases of major importance in the United States and throughout the world. Keratitis, which results from infection by herpes simplex virus (HSV) has an incidence of between 300 and 500,000 cases a year with a high level of morbidity associated with such attacks.¹ After one ocular infection of ocular dendritic keratitis, between 25 percent and 50 percent of patients will have another attack within two years² with the risk increased if the patient has had two or more attacks.

Current treatment of recurrent herpes simplex virus infections of the cornea is through use of antiviral drugs which are administered locally during periods of active infections. The application of such drugs as preventives of the disease is contraindicated, however, except when recurrences are frequent or there is a high risk of recurrent disease.³ Topical use of the antiviral protein interferon has been proposed as a therapeutic agent, but Kaufman and collaborators⁴ have failed to note any differences in the frequency of recurrence between placebo-treated patients and those patients treated with interferon in a double masked clinical trial of herpetic keratitis. The titer of human leukocyte interferon used in these studies may have been too low (6.4×10^4 units/ml) to be effective and such clinical research investigations should be repeated with a higher potency interferon from white blood cells and/or the newer immune interferon isolated from human lymphocytes after stimulation with staphylococcal enterotoxin A.⁵

It has been previously shown by Nesburn and associates⁶ that the herpes simplex virus, after infection of the cornea, can reside in the

trigeminal ganglion of experimental animals in a latent phase between active episodes of the ocular disease.

Haschke and coworkers⁷ have examined an alternative approach to treating ocular herpes during the latent phase through the use of an antiviral drug 5-iodo-5' -amino -2' , 5' -dideoxyuridine (AIDU), which was chemically synthesized with ¹²⁵I and then coupled to horseradish peroxidase. The radioactive drug-protein conjugate was shown to be capable of being transported in a retrograde manner in the axons of the trigeminal ganglion neurons by use of radioautography after corneal injection. These preliminary drug studies offer the hope of attacking the latent virus at the level of the ganglion during periods of remission of the active disease and thus preventing possible recurrences. The above drug strategy will probably require modification, however, if the strain of the herpes simplex virus, which resides in the ganglion, is determined to be different from the strain of the virus which produced the primary ocular infection.⁸

Another form of ocular herpetic infection, which has consistently proved to be a problem for drug therapy, is disciform stromal keratitis. Corticosteroids have often been employed for their anti-inflammatory and immunosuppressive effects in cases which are recalcitrant to therapy. Recently Smolin and coworkers⁹ provided hope for successful treatment of stromal herpetic infections through their studies with vitamin A. These workers have established a chronic herpes simplex virus model in rabbits by subconjunctival injections of low doses of corticosteroids to scarified corneas inoculated with the PH-strain of the virus. Animals treated with large doses of vitamin A (100,000 IU) by intraperitoneal injection were shown to develop milder, more rapidly healing epithelial lesions and either mild or no stromal disease, when compared to untreated rabbits. Vitamin A, thus, appears to be capable either of directly causing induction of this effect or acts by counteracting the immunosuppressive effect of corticosteroids. Additional research will have to be conducted to determine whether this amelioration of symptoms can be obtained through topical treatment with the vitamin.

In the 1977 report of the National Advisory Eye Council, Vision Research--A National Plan, the Corneal Diseases planning panel said that the search for better treatment of herpes infections of the cornea represented one of the major research objectives of the Program. Particular attention in that report was directed toward encouraging development of effective therapy for deep-seated, stromal and recurrent herpetic infections. Continuous attention must be given to encouraging further development of new strategies for treatment of the various forms of this debilitating viral disease.

Trophic Influences on Corneal Epithelium

The ability to manipulate specific metabolic pathways which govern the maintenance and repair of the corneal epithelium provides a possible approach to the management of certain diseases which affect the ocular surface. Because catecholamines and acetylcholine can physiologically modulate the activities of cells and tissues in many systems, recent basic research in vision has been directed toward demonstrating and clarifying such influences

on the epithelial cells of the cornea.

The corneal epithelium has been shown experimentally to respond to catecholamines in several measurable ways. The first observations of adrenergic influences on this tissue were made several decades ago by Friedenwald and Buschke¹⁰ who demonstrated that the mitotic rate of the corneal epithelial cells of the rat is suppressed by sympathetic stimulation (e.g., fright, systemic epinephrine) and by superior cervical ganglionectomy after a delay of several hours. Recent work by Butterfield and Neufeld¹¹ have updated these observations by the demonstration that the decrease in mitotic rate following ganglionectomy is associated with beta-adrenergic stimulation of the corneal epithelium which probably results from an intraocular release of norepinephrine from degenerating nerve terminals in the corneal stroma and iris. The beta-adrenergic stimulation of the epithelial layer is accompanied by an increase in cyclic AMP levels in the cornea.

Although adrenergic stimulation has been suggested as influencing the diurnal rhythm of corneal epithelial cell division in some species, decentralization of the adrenergic nerve supply to the eye of the rabbit failed to alter this rhythm.¹² The implication from such studies is that the central nervous system does not provide the pacemaker input for the diurnal rhythm via an adrenergic innervation which stimulates corneal beta-adrenergic receptors.

In vitro, catecholamines have been shown to stimulate active chloride ion transport by the corneal epithelium of several animal species.¹³ This influence is mediated by cyclic AMP which causes an enhanced permeability to anions.¹⁴ It has been recently observed by Neufeld and his collaborators in a series of studies^{15,16,17} that repeated topical application of epinephrine to the rabbit eye results in a decrease in the density of beta adrenergic receptors on the membranes of corneal epithelial cells which results in a decreased ability of the tissue layer to generate cyclic AMP and the failure of adrenergic agonists (e.g., epinephrine) to stimulate chloride ion transport. Thus, topical epinephrine is capable of depressing the entire adrenergic pathway from the cellular receptor site to the physiological response.

On the other hand, an increase in beta-adrenergic receptor density has been noted to occur on such cells after repeated topical treatment with timolol, a beta-adrenergic antagonist.¹⁵ The chloride ion transport system, however, remains unresponsive to stimulation by catecholamines long after timolol has been cleared from the cornea.¹⁷

Recent experimental studies have also been conducted to gather evidence for a possible cholinergic pathway in the cornea. The neurotransmitter acetylcholine and its synthesizing enzyme choline acetyltransferase have been found by Mindel and Mittag¹⁸ to be in the corneas of all mammalian species except the cat. These workers have also demonstrated that surgical closure of the lids of adult rabbits for 24 hours caused a decrease in the corneal choline acetyltransferase activity.¹⁹ The degradative enzyme

acetylcholinesterase has also²⁰ been located in both the epithelial and stromal layers of the cornea; the epithelial enzyme has been identified as specific for acetylcholine whereas the stromal activity is shown to be primarily pseudocholinesterase.

Although the corneal epithelial cell layer contains both epithelial and neural elements, localization of cholinergic components have not been made to specific cell types in this layer. Denervation of the cornea failed to demonstrate a decrease in acetylcholine levels even though the substrate and its related enzymes have been demonstrated to be primarily in the epithelium.¹² A transmitter function has been ascribed to acetylcholine in the cornea but neither cholinergic receptors in the tissue of the muscarinic or nicotinic type have been detected by specific radioligand assays.²¹

Thus, though there is evidence of adrenergic and cholinergic components in mammalian corneal epithelium, each pathway lacks certain components which are presently perceived as being required. The adrenergic component encompasses receptors, an intracellular messenger (cyclic AMP) and several partially understood cellular responses that presumably may be activated by stress. Missing, however, is the source to provide either the neural or humoral stimulus. The cholinergic component contains a substance which acts as a neurotransmitter with the related enzymes for its maintenance. Cholinergic receptors and evidence of cellular responses, however, are found to be lacking in corneal tissue. More research is required to complete our understanding of these possible pathways in the corneal epithelium and to correlate these observations with the recent observation that when human corneal epithelium receives thermal stimulation, only the sensation of pain is perceived in the eye of the subject.²²

Control of Corneal Hydration and Transparency

The maintenance of transparency in vertebrate corneas represents a process which expends metabolic energy and is primarily under the control of a pump mechanism which resides in the endothelial cell layer. This energy is utilized to keep the hydrophilic stroma at a minimum of physiological hydration. If the endothelial layer is damaged, an increase in corneal thickness is observed due to an imbibition of water by the stromal tissue. The resultant swelling of the transparent stromal tissue layer ultimately results in corneal opacification.

Although the importance of the endothelium in maintaining corneal transparency has been the concern of numerous studies, the exact mechanisms of how dehydration (deturgescence) can be effected are incompletely understood. A variety of compounds naturally present either in the endothelium or in the aqueous humor which bathes this layer have been recently investigated as to their respective roles in this process.

It has been observed by Riley and coworkers^{23,24}, that the tripeptide glutathione in the reduced state (GSH) at 24uM or in the oxidized state (GSSG) at 8uM can maintain perfused corneas at a normal state of hydration

for up to 5 hours. The intracellular levels of glutathione were noted to be higher in endothelia of such corneas than in those corneas swelled as a result of perfusion without glutathione. These findings are similar in effect to those results previously reported by Whikehart and Edelhauser²⁵ using higher concentrations of the compound in the perfusing medium and point to the existence of some relationship between the tripeptide levels in the medium and its intracellular level.

An alternative role, however, has been suggested for glutathione which differs from its reported effects on corneal hydration. Hull and coworkers²⁶ have recently reported that catalase, the enzyme which degrades hydrogen peroxidase can prevent loss of endothelial function which results from light exposure of photosensitized corneas. A redox buffer system such as GSH:GSSG could be important in the elimination of free radicals which are generated in the aqueous or cell membranes of the corneal endothelium.

Anderson and Wright²⁷ have studied the role of the thiol group in fluid transport in rabbit corneas by use of S-methyl derivatives of GSH and cysteine in their perfusion medium. These investigators determined that thiol-disulfide exchanges with GSSG were not necessary to achieve deturgescence of the stroma in these preswollen corneas. It was suggested that either potential Krebs cycle intermediates derivable from either the glutamyl group or gamma-aminobutyric acid (GABA) arising from glutamate are obligatory in supporting the endothelial pump mechanism.²⁸

It is interesting to note in this regard that GABA at a concentration of 10uM has been found to be effective in the deturgescence of preswollen rabbit corneas and the maintenance of such corneas for periods of up to six hours.^{29,30}

Metabolic events which take place in the cornea exclusive of the endothelial cell layer also have potential control of transparency in the total tissue. Preliminary studies conducted by Conrad and Woo³¹ indicate that the increase in transparency observed in developing chick cornea may be casually related to the degree of glycosaminoglycan sulfation which has occurred in the corneal stroma. The degree of sulfation of the principal glycosaminoglycan of the cornea, keratan sulfate, has been previously shown by Hart³² to be regulated by the intracellular concentration of the sulfate donor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), rather than by changes in the specific activity of the enzyme keratan sulfate sulfotransferase. Conrad and Woo tested corneal homogenates derived from chick embryos of different ages for their ability to synthesize PAPS and noted a 2.5-fold increase in such enzymatic activity between day 8 and day 16 of chick development which reached a peak on day 16. This sequence of events correlated well with the onset of the process of corneal transparency in the chick which begins on day 14 of development and reaches half completion by day 16.

More research is needed into identification of natural chemical factors which may affect the functions of the corneal endothelium, both as a fluid barrier and as a metabolic pump. In humans, endothelial damage which may occur during surgery, e.g., cataract extraction or corneal

transplantation may not become clinically evident as corneal edema with loss of vision for a period of several years. Knowledge of the metabolic processes which control corneal transparency may permit the design of pharmaceutical agents which could be employed to prevent the onset of corneal edema.

Collagen Type Distribution in Cornea

During the past decade, the study of the chemistry and biology of collagen in the eye and other tissues has been influenced by the discovery of the biosynthetic precursor, procollagen, and by the realization that collagens in tissues are heterogenous. Evidence has recently accumulated that the heterogeneity in collagens results from the fact that the individual polypeptide chains observed in this group of proteins are produced at different genetic loci which are nonallelic. Structural and functional homologous proteins within the collagen family have thus become known as types.

Conrad and coworkers³³ have used the developing chick cornea as a model for studying the factors that normally regulate the ratio and number of types of collagen in a fibrous connective tissue. Chick cornea appears to be somewhat unique when compared to corneal stromata of other animal species since it contains no type III collagen (composed of three type III and alpha-1 chains) post day 7 of embryonic development as determined by specific immunofluorescent staining. Prior to day 7 of development research by other investigators³⁴ has shown that antibodies to type III collagen were found to bind to corneal epithelial cells but not to the primary chick stroma. After day 7, the primary and secondary stroma of chick cornea was found primarily to contain an abundance of type I collagen (composed of two type I alpha-1 chains and one type I alpha-2 chain).

Conrad and his associates³³ have found, however, in older embryonic chick corneas, if the corneal epithelium and endothelium are gently removed after brief collagenase treatment of the stroma, the stromal fibroblasts which remain begin manufacturing type III collagen in addition to continuing to synthesize type I collagen. A similar ability to synthesize type III collagen also appeared within a few hours in corneal fibroblasts which were released from stroma and inoculated in vitro. These observations represent the first example of a switch in type of collagen synthesized by a fibroblast population although such shifts in collagen biosynthesis have been observed in cartilage cells.

The region of the corneal stroma post day 7 of development that is devoid of type III collagen has been shown to be identical with the region that stains metachromatically for sulfated glycosaminoglycans (i.e., keratan sulfate).³⁴ Indirect evidence suggests that type III collagen in the in vivo chick embryo cornea is repressed by production of keratan sulfate proteoglycan in this tissue.³³ This polysaccharide reaches half of its maximal production in the stroma by day 7 when the cornea stops synthesizing type III collagen (see review of corneal hydration).

Type II collagen (composed of three type II alpha-1 chains) has also been reported by Linsenmayer and coworkers³⁵ to be present in the anterior parts of developing chick stroma. The protein is probably synthesized by the corneal epithelium although this activity in the latter tissue layer has so far only been shown for day 6 of development.

Yue and Baum³⁶ have examined in detail the collagens synthesized by cultures of corneal stroma cells derived from both normal individuals and patients with keratoconus. Type I collagen was found by SDS-gel electrophoresis to be present in cell layer and medium fractions of both types of cultures. This observation confirmed the earlier culture work of Stoesser and associates³⁷ with human stromal fibroblasts. In addition, Yue and Baum found the A and B chains of "type VI" collagen associated mainly with the cell layer and small amounts (approximately 10 percent of total) of type III collagen present in the medium of their cultures. However, in comparison to normal control cultures, the relative amounts of "type VI" collagen synthesized by keratoconus cultures was found to be significantly increased.

Studies on collagen synthesis in vivo generally agree with those studies performed in vitro on confluent cultures of corneal stromal cells. Freeman³⁸ has determined that type I is the main collagenous product in rabbit stroma and Davison and collaborators³⁹ have isolated "type VI" segments from calf corneas.

Endothelial cell cultures have also been examined for ability to form collagen. A single basement membrane collagen classified as type IV has been isolated by Sundar Raj et al⁴⁰ from rabbit corneal endothelium. Baum and coworkers⁴¹ have found the collagen synthesized from human endothelial cultures to be extremely heterogenous. Among all the collagenous proteins, type I collagen has been identified in both the cell layer and medium fractions of such cultures. This is in agreement with a previous study of the synthesis of Descemet's membrane in vivo by Davison and Cannon.⁴² These latter workers have shown that Descemet's membrane contains not only basement membrane collagen but also several other types of collagen, in addition to type I.

The ultimate clarification of the factors which modulate the production and establish the ratio between the various type collagens in the individual layers of cornea will be important in the future for the design of therapies for achieving diverse effects within the anterior segment of the eye such as an acceleration of wound healing or alleviation of an inherited corneal dystrophy. Better understanding of the chemical structure of the individual chains of each collagen type will aid in development of a unified hypothesis for interaction of the protein with other macromolecules within corneal tissue.

Radial Keratotomy

At its May 1980 meeting, the National Advisory Eye Council expressed grave concern that the procedure, radial keratotomy, was being adopted widely, although recent reports from foreign countries and the United States

did not provide an adequate basis on which to assure the general public of its safety and efficacy. For this reason, the Council called for research on radial keratotomy and urged restraint on the part of patients and eye surgeons until the results of such research can be reviewed and evaluated by the ophthalmological community.

The resolution on the surgical procedure as developed by the National Advisory Eye Council at the May meeting is presented below:

1. As the principal advisory body to the National Eye Institute, the Federal Government's chief source of support for vision research, the National Advisory Eye Council would like to express grave concern about potential widespread adoption of an operation intended to correct near-sightedness (myopia), a common condition that can be easily and safely corrected by the use of eyeglasses or contact lenses.

The operation, called radial keratotomy, has received widespread publicity during the last year. It involves cutting the cornea with a series of deep incisions that extend from the sclera (the white tissue surrounding the cornea) toward, but not into, the center of the cornea. The incisions are intended to be deep enough to weaken the tissue so that internal eye pressure causes the edge of the cornea to bulge slightly, thereby flattening the central portion of the cornea which improves focusing. The incisions result in permanent corneal scars.

2. The Council considers radial keratotomy to be an experimental procedure because it has not been subjected to adequate scientific evaluation in animals and humans. Recent reports on radial keratotomy from foreign countries and the United States provide an inadequate basis on which to assure the procedure's safety.

3. The Council calls for carefully controlled research on radial keratotomy to determine the procedure's effectiveness, safety, short-term and long-term side effects, and the best surgical technique. The need for research on radial keratotomy and other forms of surgical correction of refractive errors (myopia, presbyopia, astigmatism) was recognized by the Council in a statement published in the journal Investigative Ophthalmology and Visual Science in August 1979. Until the ophthalmological community has an opportunity to review and evaluate the results of such research, the Council urges restraint on the part of both patients and eye surgeons.

4. The Council therefore urges the National Eye Institute to take whatever measures are necessary to encourage research on radial keratotomy in animals, and also in humans provided patients are enrolled in scientifically designed clinical trials conducted by qualified investigators.

5. The Council strongly urges that its views on the subject of radial keratotomy as expressed in this resolution be announced to the general public, as well as to health care professionals.

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Corneal Diseases

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CATARACT

Cataracts are a major class of eye disorders, being responsible for more than half of the eye related patient hospitalizations in the United States. Cataracts can result from many conditions and it has been hypothesized that they are an end response by the lens to a broad spectrum of pathologies. To develop means for the prevention and cure of this family of pathologies, it is necessary to understand the biology of the lens in its normal state and its detailed response to insults which result in cataract.

The National Eye Institute cataract program includes studies over the broadest range of biological science. In this report we will deal in some detail with three aspects of the program: molecular biology, control of biological processes and oxidative processes. Molecular biology, the study of gene composition and expression represents the first level of biological function. New developments in this area of science have burgeoned in recent years and their application to the lens, although progressing, requires additional encouragement. Important opportunities also exist in other areas of biological control. The maintenance of homeostasis may represent the fundamental means for preventing cataract and increased efforts in understanding of biological control processes are most desirable. Cataract has been associated with lens oxidative processes and increasing efforts have been addressed to the study of means by which the lens protects itself from damage by such processes.

Molecular Biology

The mammalian lens, as a relatively simple tissue, offers an attractive system for the study of normal and abnormal cellular differentiation and development. It provides a particular opportunity to examine gene expression and morphogenesis both at the level of the isolated cell (tissue culture) and in the cultured organ. This can lead to an increased understanding of the control of crucial biological functions, and, in the lens, to better understanding of normal lens function and of cataractous processes.

A firm base already exists for exploration of the molecular biology of the lens. Detailed information is available on the ultrastructure of lens cells and of their alterations during differentiation, and tissue culture cells lines have been developed which produce major gene products.¹ In vitro differentiation systems exist in which differentiation functions and the synthesis of specific gene products are activated.² Also developed have been cell fusion and hybridoma techniques which permit the synthesis of major gene products.³ Specific antibodies have been prepared for RNA's;^{2,4} procedures have been developed for the isolation of lens RNA's,⁵ and there has already been achieved cloning of a specific lens gene.

A lens mRNA, the 14S product that codes⁶ for the A2 chain in alpha crystallin, has been found to be bicistronic⁶, coding also for a B2 alpha crystallin chain. This appears to represent the first finding of a eukaryotic mRNA which is not monocistronic. The mallard and chick delta crystallin DNAs cross hybridize well, indicating conservation of gene

sequences. Early results suggest that there are at least two delta crystallin genes in the mallard lens.⁷ Lens mRNA directed synthesis of lens crystallins has been achieved in embryonic kidney cell culture.⁸ Bovine lens tissue contains two tRNA^{phe} species, one of which is increased three-fold during epithelial cell differentiation.⁹ The two tRNA's^{phe} appear to be under separate control and induction of tRNA₁^{phe} synthesis appears to be part of the differentiation program which requires the increased synthesis of crystallins. Delta crystallin gene sequences derived from the chicken genome have been cloned for the study of their organization. Results indicate the presence of at least two non allelic delta crystallin genes with small differences in nucleotide sequence.¹⁰ Fourteen intervening sequences were found in one of the crystallin genes, the intervening sequences constituting 80% of the gene. Embryonic chick lens cells have been found to contain a GTP:mRNA₁ guanyltransferase which it is postulated may be involved in mRNA synthesis.¹¹

Thus, the knowledge and methodology exist for rapid developments in this highly important area. Although progressing, lens molecular biology is moving forward at a limited pace and the attraction of additional investigators represents an important objective of the cataract program.

Control of Biological Processes

The normal lens maintains clarity through a variety of control mechanisms that determine its biological characteristics and state. Cataract represents deviation from homeostasis, so that increased understanding of the factors which control lens processes represents an important part of the lens program.

Cell proliferation in the frog lens (but not in the rat lens) ceases following hypophysectomy. In culture medium containing the serum of hypophysectomized animals, in vivo blocked lenses remain blocked; however, they can be made to resume proliferation by the addition to the medium of bovine growth hormone.¹² Somatotropin, frog prolactin, and also the thyroid derivatives T₃ and TSH restore mitosis. It is believed that pituitary hormones act by stimulating the liver to produce somatomedin-C.¹³ The mechanism by which the thyroid derivatives restore mitotic activity is not known; however, since they are without effect in the explanted lens, it is assumed that they function indirectly through the stimulation of a second factor.¹⁴ By labeling germinative zone cells in their last DNA synthetic period in hypophysectomized frogs, it has been shown that migration of epithelial cells to the equator is stopped. After hypophysectomy, lens fiber formation also appears to cease.¹⁵ It is further reported that hypophysectomy protects frogs from the development of X-ray produced cataract; thus suggesting that the initial lesion in X-ray-induced cataract is located in the germinative zone at the time of exposure.¹⁶

These studies provide early evidence and information regarding hormonal control of developmental and perhaps repair functions in the amphibian lens. Detailed elaboration is important in learning the details of these control processes. Although no similar response was observed in one mammalian species (rat), the extension of these studies in the amphibian and also in

other mammals is important and should be continued.

Understanding of tissue differentiation, the acquisition of new properties and growth patterns is a major problem in developmental biology and represents an important area for study in the lens. During lens growth, proliferating cells from the epithelial monolayer migrate to the equator where transformation occurs and the original cuboidal epithelial cells begin the process of elongation, transfer from the surface to within the lens and ultimately to the loss of the nucleus and other organelles, to become fiber cells. In in vivo studies, it was found that the optic cup environment brings about elongation of epithelial cells, and that the presence of the neural retina is important in this process. Using epithelial cells in tissue culture with determination of beta- and gamma-crystallin synthesis as a measure of transformation, it was found that inclusion of neural retina or the use of medium previously incubated in neural retina in the culture medium led to appreciable cell enlargement and initiation of the synthesis of beta- and gamma-crystallins. Although other eye tissue, e.g. corneal stroma, also was stimulatory, its effect was appreciably smaller. Using an elegant microscope mounted cell culture technique permitting early quantitative measure of epithelial cell enlargement, Beebe¹⁸ has identified and isolated lentropin, a vitreous factor in the chicken eye having a molecular weight of approximately 60,000 daltons which promotes fiber cell differentiation. Also, marked stimulation of DNA synthesis in a cloned Nakano mouse epithelial cell line was found by a factor secreted by a human retinoblastoma cell line.¹⁹ Again, evidence has been obtained which indicates that lens cell elongation results from an increase in lens cell volume and not from the influence of microtubules as had been previously believed.²⁰ Using chick embryo lenses, it has been shown that four events which occur during fiber cell differentiation, namely, cell elongation, cell division, differential synthesis of delta crystallin and accumulation of delta crystallin mRNA can be experimentally uncoupled.²¹ Hence, each event is under independent control. Further, four differences have been identified between 15 day embryonic chick lenses cultured with their vitreous bodies and those cultured without them.²² These differences include: delta crystallins synthesized in embryonic chick lenses without vitreous bodies have higher isoelectric points, there is an alteration in the ratio of synthesis of the two sizes of delta crystallin peptides, a change in the intracellular concentrations of sodium and potassium and in the formation of inter- and intracellular vacuoles.

Biological control of the various lens processes and the means by which homeostasis is achieved and maintained represents a central problem in lens and cataract research. It is an area of study that deserves increasing emphasis.

Oxidative Processes in the Lens

Oxidative and peroxidative systems have long been implicated in cataractogenesis and recent evidence gives increasing support to this view.²³ The cataractogenic agent, 3-aminotriazole, a specific inhibitor of catalase, when fed to weanling rats causes a marked increase in hydrogen peroxide level in the aqueous humor. The latter increase in turn acts to inhibit

superoxide dismutase, the enzyme which catalyses the reduction of the highly reactive superoxide radical, O_2^- . X-Irradiation causes a reduction in the levels of various lens reducing systems.²⁴ At the time of cataract development, several weeks after X-irradiation, there is a sudden fall in protein-SH groups. However, subsequent to irradiation and prior to cataract formation, there is reduction in glutathione, in NADPH, in hexose monophosphate shunt activity and glucose-6-phosphate dehydrogenase. The results indicate a steady decrease in the effectiveness of lens reducing systems and it is suggested that when these enzymes reach a critically low point, there is sudden oxidation of protein-SH, with formation of high molecular weight aggregates. Another effect of X-irradiation is alteration of lens DNA. Since peroxide scavengers provide a protective role, it is suggested that the mechanism for alteration is oxidative in nature.²⁵ An increase in the number of lens protein disulfide bonds has been reported in human senile cataract, diabetic cataract and in several experimentally induced cataracts. In general, the increase in disulfide parallels severity of cataract. In the aging normal lens there is increased oxidation of cysteine and methionine. However, the increased oxidation is restricted to membrane-contained or -associated components. In the senile cataractous lens, increased oxidation spreads to cytoplasmic components.²⁶ Evidence exists for the peroxidation of membrane lipids in human cataractous lenses.²⁷ Studies on the lens in tissue culture of the effect of peroxidants and antioxidants on membrane ion transport give support to the importance of reducing systems in protecting lens functions.²⁸ Thus, visible light irradiation of lens in riboflavin containing medium (to produce oxidative free radicals) markedly reduces transport function. Addition of superoxide dismutase or catalase provides protection against the damaging irradiation. It is suggested that the principal agent for lens damage in these experiments is the superoxide radical. Addition of ascorbate similarly provides protection, and it is suggested that this may be the role for the high level of ascorbate in the aqueous humor. Peroxidative action also affects lens cation transport and other biological activities. Thus, cultured rat lenses show decreased choline accumulation when irradiated in the presence of riboflavin or methylene blue. In the presence of catalase, choline levels rise to near normal.²⁹ Photo-peroxidation of cultured animal lenses causes the production of malondialdehyde, a product of oxidative lipid breakdown,³⁰ which parallels related findings in human cataractous lenses.²⁷ Subcutaneously administered sodium selenate causes cataract in rats. The concomitant increase in peroxide level in the aqueous humor together with reductions in catalase and superoxide dismutase activities and in protein thiol levels suggest an oxidative or peroxidative mechanism in development of this cataract.³¹ In a methylene blue sensitized photooxidation system, involving singlet oxygen (1O_2), lens crystallin polypeptides become crosslinked and develop a blue fluorescence similar to that found in the aging human lens.³²

Oxidative processes can cause complex changes in biological systems. These may include molecular weight increases in proteins through crosslinking, changes in protein tertiary structure through modifications of component amino acids and a variety of other transformations. Lipids and also carbohydrates and nucleic acids are subject to complex oxidative changes. In its normal state, the lens seems able to overcome these potentially

damaging reactions. It is important to understand the details of the processes that protect the lens from oxidative damage and to learn which of the potential damaging reactions may be related to the development of cataract.

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Cataract

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Introduction

Glaucoma encompasses a group of diseases in which loss of vision is correlated with an increase in intraocular pressure (IOP) and changes in the color and dimensions of the optic cup. If detected early, a large percentage of primary open angle glaucoma cases can be successfully treated over long periods of time by current medical and surgical means, while some of the types of glaucoma occurring secondarily to other diseases or to trauma are often quite intractable. Acute angle closure glaucoma, if treated promptly, can be managed successfully; however, a reliable means of predicting attacks of angle closure is still needed.

Research objectives for the prevention, diagnosis and treatment of glaucoma were set forth in the National Eye Advisory Council's Vision Research--A National Plan: 1978-1982. Areas signaled for attention for research to gain needed knowledge of the different kinds of glaucoma included: 1) etiology of the various types of glaucoma, including studies on genetics, prognosis, and mechanisms of the disease processes; 2) optic nerve damage and vision changes, documenting changes in optic disc and its relationship to increased intraocular pressure and blood pressure, and searching for improved early diagnostic and prognostic information; 3) aqueous hydrodynamics, the factors controlling secretion and outflow of aqueous humor and thereby controlling intraocular pressure; and 4) medical and surgical treatment, including studies of drug action, clinical trial tests of promising drugs, and evaluation of laser surgery.

For this report, research projects were selectively chosen which bear upon some of these objectives. These topics are of especial interest because they provide refined definitions of the ocular tissues and drugs which act upon them, and highlight potential applications of new types of instrumentation and drug delivery. The first section, Aqueous Humor Hydrodynamics, deals with studies involved in describing the structure and pharmacology of the main fluid outflow pathway from the anterior chamber and with drugs affecting aqueous humor secretion. The second section, Adrenergic Pharmacology, examines in some detail the interactions of drugs and endogenous substances modulating adrenergic reactions with ocular tissues, as they relate to normal physiology and therapy; some of this data is complementary to that in section one. A short third section deals with other endogenous substances or drugs, a peptide neurotransmitter and arachidonic acid derivatives, which may have significant effects in normal functions, in ocular inflammatory conditions, and in controlling intraocular pressure. New methods for measuring the structural dimensions and appearance of the optic disc and recording such data and a possible test for early detection of optic nerve damage are noted, and results of applied investigations are discussed. Finally, early results of a study of a promising drug delivery system and a summary of an investigator's extensive experience with argon laser surgery in treating specific patient population are presented.

Aqueous Humor Hydrodynamics

1. The Outflow Pathway: Structure

The studies of Alvarado, Wood and Polansky, defining the properties of several lines of cultured normal human trabecular meshwork cells, are continuing.¹ Cells cultured for up to four passages have been compared with freshly fixed cells of intact tissue. Trabecular cells in culture grow as a single cell type, morphologically distinct from corneal keratocytes and corneal endothelial cells and other potential cellular contaminants, and are similar morphologically to cells of dissected trabecular meshwork which also appear to be a single cell type. Electronmicroscopic comparisons showed that the intracellular organelle organization, specialized cell surfaces, and intracellular junctions of cultured cells and cells in intact tissue were similar. Cultured cells secreted intercellular materials similar to those seen in intact tissue, including collagen-like filaments of 110A° periodicity and amorphous substances including fibronectin. These passaged cells also showed phagocytic activity and incorporated particles into organelles having acid phosphatase activity (probably lysosomes). Trabecular cells of both intact and cultured tissues were non-thrombogenic, further differentiating them from keratocytes. Therefore, trabecular cells in both states represent a single cell type having fibroblastic, endothelial and phagocytic properties, and they secrete fibrous and amorphous connective tissue elements. (Secretion of glycosaminoglycans has been shown previously).

In an effort to determine if the ultrastructure of the outflow region in eyes of glaucoma patients differs from normal, Chaudhry et al.² made scanning electronmicroscopic observations of trabeculectomy specimens. The meshwork of several open-angle glaucoma patients appeared to be markedly different from normal tissues, having sheet-like depositions obstructing the trabecular meshwork to varying degrees. The investigators have ruled out fibrin deposits as an artifact in producing the sheets. Over the course of many years it has been suggested that blockage of the meshwork by glycosaminoglycans or proteoglycans might cause glaucoma. It should be possible, by coordinating enzymatic and histologic work with SEM studies, to determine the composition of deposited material, if it is normally a component of trabecular meshwork, and if it is present in abnormal amount in the angle in glaucoma.

2. The Outflow Pathway: Glucocorticoid Effects

Because glucocorticoids (GC) induce an initially reversible glaucoma accompanied by reduced aqueous outflow in certain susceptible people, and glaucoma patients may have enhanced hypertensive responses to GCs, much effort has been concentrated on examining the effects of GCs on the outflow pathway, and to determining if there is a generalized cellular sensitivity to GCs in glaucoma-prone individuals which would aid in prognosis of the disease. Hypotheses that there were simple correlations of GC sensitivity or heritable cell antigen types with incidence of glaucoma have not been confirmed. Several studies of GC effects on readily available cells, circulating lymphocytes and skin fibroblasts, have yielded contradictory reports of significant differences in cellular sensitivity to GC between

glaucoma patients and normal persons (testing GC inhibition of mitogen induced cell transformation); more recent work has showed that each of these cell types from both groups of people has similar numbers of GC-binding sites and similar binding affinities for GCs. Nonetheless, it is still important to understand the cellular basis of specific ocular tissue reactions to glucocorticoids.

A careful re-evaluation of the corticosteroid inhibition of the mitogen induced lymphocyte transformation reaction in Schwartz's laboratory showed that there was no significant difference in response among cells or serum from normal individuals, ocular hypertensives, and open angle glaucoma patients.³ Cells were cultured both in serum-containing and serum-free media to rule out possible serum modulators which might be the true determining factors in the reactions. Also, there was no significant difference in plasma cortisol levels between the groups. Palmberg re-examined the GC inhibition of cultured fibroblast growth and found no difference in responses of cells from normals and open-angle glaucoma patients.⁴ The reaction is non-specific because the amounts of GC needed for 50% inhibition of fibroblast growth were 3-4 orders of magnitude greater than required to saturate specific GC receptors. Palmberg's data also suggest that the original observations of differences in cellular sensitivity were inadvertently biased by patient selection, many patients being tested while under the stress of hospitalization or surgery. Therefore, there is no generalized cellular reaction of lymphocytes or fibroblasts with glucocorticoids capable of easily differentiating glaucoma patients from normal persons, and explanations for the ocular hypertensive effects of GCs must be sought in ocular, not peripheral tissues.

Tchernitchin et al⁵, using an autoradiographic technique, found that ³H-dexamethasone was specifically localized in nuclei of stromal and endothelial cells of the outflow region, indicating that GCs may also play a specific role in cells of the aqueous outflow pathway, possibly by modulating outflow facility.

3. Aqueous Secretion: Glucocorticoid Effects

The search for glucocorticoid effects on aqueous humor hydrodynamics is shifting to studies of GC reactions with ocular tissues. Recently, Southren et al demonstrated specific binding of glucocorticoid to rabbit iris-ciliary body receptors and showed that enzymes involved in GC metabolism are present in these tissues. Further investigation now shows that these GC receptors act in response to hormonal stimulation as do the well characterized GC receptors in other cells.⁶ GC receptors are located in the cytosol and, when bound to GCs, translocate to the cellular nucleus where their primary biologic effects are exerted. Specific binding of cortisol to iris-ciliary receptors was measured, and the amount of receptor labeled following injection of tritiated cortisol was determined in the cytosol and nucleus. Within 30 minutes, the amount of cytosolic cortisol diminished by about 66% while nuclear cortisol content increased to nearly the same extent. The trans-location mechanism was limited to glucocorticoids, the sex steroids being inactive in this process. If iris-ciliary tissues have specific cytosolic receptors for glucocorticoid and bind GC to nuclei following translocation, then GCs must have specific

functions in this tissue. It remains to be seen if this function bears directly on control of aqueous humor secretion or relates to other cellular metabolic processes.

4. Aqueous Humor Secretion: Carbonic Anhydrase Inhibitors and Carbonic Anhydrase

The clinical use of presently available carbonic anhydrase inhibitors (CAI) is severely limited by their considerable number of undesirable side effects. The drugs must be administered orally, necessitating high dosages which effect organs apart from the target ciliary secretory cells. Two recent reports indicate that effective topical drugs may soon become available. Maren and Scott have each reported that a topically applied effective analog of acetazolamide (Diamox), bromacetazolamide, markedly reduced IOP in rabbits and reduced aqueous flow in cats. Bromacetazolamide is an alkylating non-reversible CAI, which is markedly more soluble in water than acetazolamide. Good corneal penetration because of increased solubility, rather than irreversible binding, is responsible for its effectiveness. Another similarly promising drug is trifluormethazolamide. Features common to both drugs, differentiating them from acetazolamide are that the free acids are quite soluble in water, have some lipid solubility, and have sodium salts that are soluble at physiologic pH values.

The enzyme carbonic anhydrase (CA) plays a significant role in production of aqueous humor by the ciliary body. There are several enzymes, isoenzymes, with CA activity. The nature of the carbonic anhydrase of ciliary processes is still not well defined. Maren has discussed the requirements of the physiologic activities affected by CA activity, primarily the need for directional secretion across membranes. Such a vectoral process should be mediated by membrane-bound enzymes or carriers. In kidney membranes, involved in a secretory process, the CA (MCA) is distinct kinetically and immunologically from the cytosolic isoenzymes in red cells. The MCA represents about one-third of total kidney CA activity, and the concentrations of acetazolamide used in therapy inhibit it 99.9%. Based on the kidney data, Maren suggests that an analogous MCA in ciliary processes, rather than the cytosolic enzyme, should be the significant factor in aqueous humor secretion, and that inhibitors selective for it would be effective drugs for treatment of glaucoma.

Scott has analysed the distribution of CA activity in rabbit ciliary process homogenates. About 35% of total tissue CA activity was found in membrane extracts. The membrane-bound CA had a higher specific enzymic activity than the soluble enzyme. Following topical application of bromacetazolamide to rabbit eyes, total ciliary CA activity was lowered, and both soluble and membrane enzymes were inhibited (no data on IOP was reported). On the other hand, a cytosolic CA in human ciliary processes, antigenically similar to the high activity erythrocyte enzyme, was reported to be the sole CA of ciliary processes.¹⁰ Scott speculates that his membrane extraction procedure was more gentle than that used in the other studies.

In an effort to determine if differences in individual human responses to carbonic anhydrase inhibitors were attributable to different CA isoenzymes, Dobbs et al measured the inhibition kinetics of CA from ten pairs of eyes.¹¹ Only a single isoenzyme was detected (identified as the cytosolic enzyme), suggesting that differences in human responses to CAIs must be due to other factors than the type of soluble CA in the ciliary body. Since only normal eyes were tested, there is still a possibility that a different isoenzyme or distribution of isoenzymes occurs in eyes susceptible to glaucoma.

Carbonic anhydrase inhibitors have been in use for over 20 years for treating glaucoma, but their mechanism of action in controlling IOP has not been well-defined. The work summarized above represents some new approaches to gaining knowledge of the properties of the enzymes involved and their locations. The fact that available drugs have only been effective orally has limited their use, and had led to the idea that no topical drug would be an effective CAI inhibitor. The studies with bromacetazolamide and trifluormethazolamide re-open the question and may lead to the development of a useful class of topical CAIs.

Adrenergic Pharmacology

The mechanisms of action of both the endogenous adrenergic neurotransmitters and adrenergic drugs affecting aqueous humor dynamics are still poorly defined. Re-examination of commonly held ideas is providing new insights about the actions of these drugs that may have practical benefits in treating glaucoma.

As with most biologically active molecules, adrenergic drug effects are usually stereospecific, the l-isomers having predominant biologic activity over d-isomers (for example: as enzyme substrates in rates of their neuronal uptake, and in exerting cardiovascular effects). On examining the ocular hypotensive effects of stereoisomers of several adrenergic drugs, Rowland and Potter found that several d-isomers had ocular hypotensive effects in rabbits equal to, greater than, or less than those of the l-isomers.¹² For example, the d- and l- isomers of epinephrine (EPI) and isoproterenol had about equal hypotensive effects, the d-isomers of norepinephrine (NE) and soterenol were more potent than the l-, and the l-phenylephrine isomer was the more potent of its pair. The degree of mydriasis was also sterically determined for each of the drug pairs, but the relative directions and intensities of response did not entirely correspond to those of the hypotensive activity. These findings should provide tools for better understanding the mechanism of action of the adrenergic drugs in controlling ocular tension. Also, since the d-isomers do not have significant effects on cardiac or vascular tissues, these observations may lead to either direct therapeutic applications or to the development of improved drugs for treating glaucoma.

Reactions of beta (B)-adrenergic drugs or neurotransmitters at their receptors are often linked with activation of adenylyl cyclase (AC) to produce cyclic adenylic acid (CAMP), the "second messenger", which modulates target cell reactions. Coupling the receptor reaction and the AC activation reaction

is a guanyl regulatory protein (GRP), itself activated by guanine nucleotides. Functionally, the three proteins are probably associated in a cell membrane complex. Detailed investigations of drug reactions with ocular tissues based upon these newer general pharmacologic findings will help to explain normal cellular controls of aqueous hydrodynamics and to predict how specifically tailored drugs should act in glaucoma therapy.

Mittag has measured cyclic adenylate in aqueous humor at intervals following topical administration of epinephrine to rabbit eyes.¹³ CAMP levels rose quickly, coinciding with a rise in IOP during the first hour, and peaked by 5-6 hours, at which time the prolonged ocular hypotensive effect of EPI was just beginning. Timolol (TIM) blocked the CAMP increase, but had no effect on the EPI-induced fall in IOP, apparently dissociating any CAMP-associated beta-receptors from the hypotensive reaction. Since the effects of most adrenergic reactions occur and decay rapidly, there is a question as to whether CAMP-associated B-adrenergic reaction is directly involved in the hypotensive reaction. It seems that rather than activating B-receptor reactions to modulate IOP, topical EPI must restrict either adrenergic receptor numbers or their availability, causing a functional subsensitivity. Mittag hypothesizes that EPI, in affecting the delayed long-term hypotensive response, acts as a B-receptor desensitizing agent, not as a receptor agonist. The relationship of guanyl regulatory protein to the EPI ocular reactions was also studied. Tissues from eyes pre-treated with EPI produced less CAMP than controls when either guanyl nucleotides alone or in combination with B-adrenergic agonists were used, suggesting that EPI uncouples or desensitizes the activation of AC by GRP and decreases activity of the B-receptors which modulate the AC-GRP interaction (and thereby decreases the beta-physiologic response). This complements the finding of Neufeld¹⁴, that the density of B-adrenergic receptors (i.e. availability of receptors, measured by binding of radioactive reagents) was inversely related to the level of adrenergic stimulation. In vitro, B-adrenergic-stimulated AC activity of iris-ciliary homogenates from rabbit eyes pretreated with topical EPI was significantly less than that from control eyes, again suggesting that efficacy of glaucoma treatment depends upon desensitization of B-receptors. This observation may help to explain why B-agonists and antagonists both lower IOP, the agonist (EPI) acting functionally as a partial antagonist in diminishing receptor numbers or availability.

The data of Woods and Waitzman is also suggestive of drug induced receptor changes. Here, one dose of drug modified receptor responses to subsequent doses of the same drug.¹⁵ The course of IOP changes in rabbit eyes was monitored following spaced topical doses of NE. The spacing of dosing intervals actually determined the nature of the response. A second dose of NE at two days produced the usual biphasic effect on IOP (a short early hypertensive phase followed later by a longer hypotensive phase); however, if the second dose of NE was given at seven days, only the hypotensive effect was seen, and IOP fell to a lower level than produced by the first dose. This observation may have a practical therapeutic application; perhaps adrenergic agonists would be more effective if administered on a pulsed schedule rather than on a constant rate basis.

These investigators also treated rabbit eyes with guanylylimidodiphosphate (GIDP), which reacts with the regulatory protein GRP to activate AC, and produced a significantly reduced IOP.¹⁵ However GIDP, administered concurrently with NE, produced a less than additive fall of IOP. When the eyes were pretreated with NE 2 days before addition of GIDP, a somewhat delayed hypotensive response occurred which endured to twenty hours. In brain, GIDP can hasten the reactivation of receptors after short-term inactivation. This data is consistent with the other observations that it is inactivation of B-receptors on secretory ciliary epithelial cells that is responsible for lowering aqueous production. Pretreatment of eyes with fenclorac (FEN), a reversible PG inhibitor, which alone had no appreciable effect on IOP, eliminated the NE-induced hypertensive phase and delayed the onset of the ocular hypotensive phase.¹⁵ Together, these findings indicate that the B-receptor-AC-GRP complex modulates IOP, and that selective pharmacologic interventions should offer means of controlling IOP; however, the cells involved in these reactions first must be identified.

Several investigators have analyzed the effectiveness of mixed adrenergic drug therapy on humans or animals. The lowering of IOP by drugs having oppositely defined actions, EPI, an agonist, and TIM, an antagonist, seems paradoxical. The following investigations provide some insights into how these drugs may act, and whether, as some believe, they truly are effective in combination. This is a practical clinical problem encountered in treating patients with poorly controlled IOP.

In an acute experiment, Higgins and Brubaker treated normal human eyes with timolol, then epinephrine was administered.¹⁶ TIM alone lowers IOP by reducing aqueous humor formation. EPI, is known to act at two sites, increasing aqueous formation to a lesser extent than it increases outflow rate, the net effect being increased outflow. When EPI was administered after TIM, aqueous formation was decreased by an additional 7% (significant). It is possible that EPI displaced TIM from outflow sites which it blocked without exerting any effect.

Goldberg et al pretreated both eyes of glaucoma patients for a week with either epinephrine or timolol.¹⁷ Then a single drop of the other drug was administered to one eye, and IOP was monitored for six hours. Pretreatment with TIM considerably reduced the hypotensive effect of epinephrine compared to that caused by EPI alone, but when the drugs were used in reverse order, EPI failed to increase the IOP reduction caused by TIM. A generally accepted explanation for why two drugs have an additive effect is that they act upon a common physiologic process at different cellular sites. If EPI initially increases outflow rate, and TIM decreases production of aqueous, the two drugs would synergistically exert their effects at different points in the system to lower IOP. Therefore, it appears that TIM given first must occupy, without effect, B-sites on outflow pathway cells that otherwise would be responsive to EPI while on a short-term basis, at least, if EPI is given first, it is not rapidly displaced rapidly from these sites by the antagonist TIM, and TIM also exerts its action at the second, independent aqueous secretion site.

A long term clinical experiment conducted by Thomas and Epstein also has analyzed the course of mixed drug therapy with EPI and TIM, and has led to similar conclusions.¹⁸ Using a double-masked design, glaucoma patients were maintained on either TIM or EPI for two weeks, then the other drug was added to the regimen. In each case, an initial further decrease in IOP was noted; however, during six weeks of continued mixed drug therapy, the additive effect of the drugs was gradually lost. Depending upon the order of drug administration, the nature of the responses differed, the initial additional fall in IOP being significantly greater when TIM was added to EPI dosage than vice versa. Further, while the initial effect of adding TIM to EPI was to increase facility of outflow, after two weeks there was a decrease; in the reverse case, adding EPI to TIM caused no change. The following hypothesis was proposed, suggesting a mechanism for controlling IOP involving B-receptors in both aqueous inflow and outflow systems: TIM blocks an EPI effect on outflow, previously thought to be controlled by an alpha-adrenergic mechanism. Beta-receptors on trabecular meshwork cells function without tone, so while stimulation by EPI increases outflow, antagonism by TIM is without effect. At the secretory ciliary epithelium, presumed to have tone,¹⁴ TIM inhibits the aqueous production stimulated by EPI (possibly by limiting blood flow). And finally, the loss of additivity with time is due to TIM gradually displacing EPI from beta receptors of trabecular meshwork cells. The transient enhanced reduction in IOP caused by adding TIM to EPI therapy suggests that if patients on EPI require additional treatment, that pulsed administration of Tim should be more effective than continuous combined drug use.

Akthar and Abdel-Latif have continued to explore²¹ the role of phospholipids in the neuromuscular reactions of iris muscle. In a previous study, they found that the reaction of iris muscle alpha receptors with NE stimulated the shift of phosphate from triphosphoinositol (TPI) to phosphatides, and calcium ion flux appeared to link the two processes. In continuing studies with iris muscle, acetylcholine acting at muscarinic receptors increased calcium uptake and decreased its efflux and also increased TPI metabolism; in contrast, the alpha-adrenergic reaction of the muscle receptors with NE increased calcium efflux and decreased its uptake. In both cases, the reactions of neurotransmitters with iris led to a breakdown of triphosphoinositide. Also surgical sympathetectomy or electrical stimulation of the sympathetic nerves to the iris induced receptor supersensitivity to NE and increased the TPI effect. The investigators postulate that cellular phosphoinositides play a modulating role in the pathway linking iris muscarinic cholinergic and alpha-adrenergic receptor reactions to muscle contraction via transcellular movement of calcium ions.

Clearly, much remains to be defined about how neurotransmitters and drugs affect aqueous humor dynamics, and many commonly held assumptions can stand re-examination. The work noted here suggests that in clinical use, drug dosage scheduling is a very significant determinant of efficacy in control of IOP. Also, as the cellular basis of control of IOP is defined, new drugs such as those acting upon the B-receptor-adenyl cyclase-guanyl regulatory protein system, may be useful.

Non-adrenergic Pharmacology

Substance P (SP) is a peptide becoming of increasing interest as a mediator, or possibly a neurotransmitter, in sensory nerves. SP is released in peripheral nerves following electrical stimulation and causes signs of inflammation; electrical or mechanical stimulation of ocular sensory nerves also causes miosis and inflammation (not prostaglandin induced). Bill et al found that stimulation of the rabbit trigeminal nerve caused release of a material into the anterior chamber which was identified immunologically as SP.¹⁹ Further, intracameral injection of SP caused miosis and a breakdown of the blood-aqueous barrier. Therefore, SP must have a role in the sensory nerves of the eye similar to that in other nerves. In another type of experiment, Camres and Bito demonstrated the presence of SP in the eye and indicated that it may be the primary mediator of immediate ocular responses to chemical irritants and surgical trauma.²⁰ Three days after pre-treatment of rabbits with capsiacin, which depletes primary sensory neurons of SP, topical administration of the irritant nitrogen mustard failed to increase IOP or change pupil diameter, whereas in the absence of capsiacin both factors increased significantly. Treatment with indomethacin failed to prevent these early changes, again indicating that prostaglandins were not involved. Also, an SP-like substance in iris-ciliary body was detected immunologically and it was reduced in amount by 50% following mustard application.

Arachidonic acid derivatives, the prostaglandins (PGs), thromboxanes (TX), and prostacyclin (Pgl) are proving to be important in modulating many physiologic processes. Most of the major known compounds have now been identified in iris-ciliary body (noted in the 1978 NEI Annual Report: Waitzman and Woods; Colasanti and Barany; Kulkarni et al; Eakins and Banerjee), but their precise functions in anterior segment physiology remain to be defined. Prostacyclin and thromboxanes play important and generally opposing roles in many processes; for example, Pgl inhibits platelet aggregation, is a vasodilator and relaxes smooth muscle, while TXA₂ exerts opposite effects in these systems. TXA₂ contributes to inflammatory responses and Pgl may have an anti-inflammatory function. Prostaglandins are also implicated in some adrenergic reactions controlling normal physiologic processes and responses to drugs, so their roles in normal and abnormal ocular function need to be elucidated. Non-steroidal anti-inflammatory drugs such as indomethacin inhibit the production of all of the PG, Pgl and TX products; however, imidazole inhibits only TX synthesis, which may allow for its selective use in the study and treatment of inflammatory reactions.

The effect of various pharmacologic agents on the synthesis of the physiologically active arachidonate derivatives is under active investigation. An important first question is the distribution and availability of arachidonate for PG synthesis in ocular tissues. Little is known about the storage form of arachidonate in iris; in non-ocular tissues it is stored esterified in glycerolipids, and the rate of arachidonate release from its storage esters is rate-limiting in prostaglandin synthesis. Iris muscle actively synthesizes prostaglandins and Abdel-Latif and Smith have now shown that ¹⁴C-arachidonate is taken up by rabbit iris, both in vivo and in vitro,²² and have determined in what compounds it is stored in glycerolipids.

In rabbits, topical administration of PGE₂ increases intraocular pressure and non-steroidal anti-inflammatory agents can prevent its rise. Dipyridamole, which inhibits TXA₂ biosynthesis,²³ was used by Podos to pretreat rabbits prior to PGE₂ administration. Systemic, but not topical, dipyridamole was effective in preventing the PGE-induced increase in IOP. Dipyridamole is also a CAMP-phosphodiesterase inhibitor (as is imidazole) and a second mechanism of action may be at the level of CAMP-mediated reactions since prostacyclin stimulates CAMP synthesis in other tissues. Therefore, dipyridamole may act by shifting the balance of physiologic reactions mediated by the thromboxane-prostacyclin pair.

There is much interest in the potential of marihuana and its constituents or derivatives for treating glaucoma. Smoking marihuana lowers IOP in some normal and glaucoma patients, but its long-term safety and efficacy in glaucoma therapy remain to be defined. Merritt monitored its effects on glaucoma patients after they smoked marihuana cigarettes.²⁴ Ocular hypotensive effects were observed within 60-90 minutes, and were accompanied by systemic hypotension. Severity of side effects (cardiovascular and perceptual) was judged to preclude further clinical use of inhaled marijuana combustion products in a glaucoma patient population. Marijuana's mechanism of action in affecting aqueous humor hydrodynamics is not understood. Krupin and colleagues²⁵ have shown that, in vitro, anterior rabbit uveal tissue accumulates delta 9-tetrahydrocannabinol (THC, an active component of marijuana) against a gradient in an energy dependent manner. In vivo, following intravitreal injection, THC exited from uveal tissues far more rapidly than the comparably sized sucrose molecule, again indicating an actively directed process. THC was not appreciably metabolized in the eye. Kinetics studies indicated that accumulation of THC by the uvea is an active, carrier-mediated process. Since THC lowers IOP in many eyes, the finding that iris-ciliary body tissues actively accumulate and lose it is a first step toward defining its mechanism of action. More controlled clinical studies will be required to determine if marihuana or any of its extractable components will have a useful role to play in glaucoma therapy.

Instrumentation and Methodology: Visual Field and Optic Disc Properties

Accurate measurement and documentation of visual field data and optic nervehead morphology and comparisons of longitudinal changes in these measurements are important in determining the efficacy of glaucoma treatment and in predicting and detecting the progression of ocular hypertension to glaucoma. Indeed, there is hope that more exact measurements will reveal subtle changes that will allow for earlier diagnosis and prognosis than is presently feasible. The development of computerized controls for laboratory instruments and simpler, relatively inexpensive computer data storage and retrieval systems is leading to developments immediately applicable to treatment of glaucoma and to conducting accurate prospective studies of ocular hypertension.

Hartz, Hart and Blaine²⁶ have been concerned with means of collecting, storing, recalling, and analysing visual field data, and have described a micro-processor-based system for use with kinetic Goldman perimetry. In

this model, the perimetrist operates interactively with the data acquisition system. The coordinates from the instrument's printing pantograph are recorded via a sound-detecting triangulation system and are immediately displayed as visual field contours on an oscilloscope. This allows for repeating ambiguous measurements if necessary, and editing. When the operator is satisfied with the displayed data, it is converted to digitized form suitable for mini-computer storage and is available for later selective analysis and comparisons.

A somewhat different and less complex approach to acquiring and managing visual field data has been taken by Dueker²⁷. Here, field contours, often outlined in color, are conventionally recorded. Records are scanned by a videocamera, using color filters, that acts as an input to a computer system which digitizes the contour data and stores it for recall and analysis.

Shapiro has devised a number of instruments and controls to aid in his studies of optic nervehead morphology and optic disc circulation.²⁸ The instruments which are described in a number of engineering publications (not cited), include: an optical system to compensate in three dimensions for eye movement during rapid fluorescein angiography (including mathematical models, mechanical designs, electronics, and computer programs) and two systems to measure eye position and eliminate instrument drift, one using ERG signals on skin near the canthi with appropriate electronic amplification and filtration devices, the other a magnetic sensor which uses a probe on a contact lens. With the improved instruments and computerized analysis techniques, the laboratory measurements can be taken and processed in minutes rather than hours, as was the case a few years ago. For example, the effects of chronically increased IOP in rhesus monkeys (averaging 20 mm over normal following laser photocoagulation of the trabecular meshwork) on optic nervehead topology and circulation were examined. Delayed disc filling, recorded by rapid sequence fluorescein angiography, was observed, particularly on the nasal side. The effects of chronic systemic hypertension on these parameters were measured on the eyes of 26 cyanomolgous monkeys (maintained by another investigator), and no significant circulatory differences were found in comparison with the eyes of normotensive monkeys. Laser contour angiography was used to measure the effects of chronic high IOP on the disc dimensions. As individual eyes progressed from the normotensive to the hypertensive state, statistically significant 2-3-fold increases in cup/diameter ratios, cup volumes and cup depth were measured. These findings indicate that circulatory changes of the optic disc are more related to increased intraocular pressure than to systemic hypertension.

Because some ocular hypertensives will progress to visual field loss if not treated to reduce IOP whereas others may never suffer significant loss of visual acuity, there is a great need for accurate predictive tests to differentiate "benign" ocular hypotension from that with a high probability of progressing to glaucoma. For maximum value, a battery of tests should measure as many independent variables as possible. Retrospective and prospective studies monitoring changes in visual fields, optic cup dimensions and disc pallor, are seeking correlations specific to development of glaucoma. A

wider range of prognostic aids, based upon independent biologic or psychophysical measurements would aid considerably in the differentiation process. It has been suggested that color sensitivity loss or changes in contrast sensitivity may precede measurable visual field changes as glaucoma develops. Atkin et al²⁹ have investigated a test of central contrast sensitivity as a possible diagnostic aid. The test is based on two types of stimuli presented simultaneously--diffuse flicker and counterphase flicker. For each stimulus presented alone, contrast sensitivity means were lower for glaucoma patients than for controls; however, there was considerable overlap of data from individuals in the two groups. When the average of values of the two test results was used (DRC or dynamic response coefficient), the glaucoma group was distinguishable from the control group at a statistically significant level. Five of ten ocular hypertensive eyes had DRC values that fell into the "glaucoma" range. In none of the patients was there a clear relationship between IOP (or use of medication) and DRC. These preliminary results indicate that it may be possible to detect an early signal of glaucoma prior to measurable loss of visual field, to initiate early therapy of vulnerable ocular hypertensives and to spare the need for treating "benign" hypertensives. It will be of considerable interest to see how these findings fare in expanded and in longitudinal studies.

A drug delivery system

Liposomes, lipid microdroplets enclosing another solution or suspension, present a theoretically useful means of drug delivery. If drug-containing liposomes can attach to the corneal epithelium and degrade slowly, releasing microamounts of a topically administered drug directly into epithelial cells from which it can proceed into the anterior chamber, they may prove to be a useful tool for anti-glaucoma drug administration. Schaeffer, Klug and Krohn have shown that positively charged liposomes adhere to cornea more effectively than negatively charged or neutral ones.³⁰ The trans-corneal flux into the anterior chamber of certain free and liposome-bound drugs was measured. Test drugs included penicillin G, an antibiotic, and indoxole, an anti-inflammatory agent. Empty liposomes plus free drug or free drug alone were used as experimental controls. Measurements of drug concentrations sixty minutes after application showed that penicillin concentration in rabbit aqueous humor was increased five-fold over controls, and that indoxole concentration was increased ten-fold over controls in rat aqueous humor. Thus, this may be a means to administer drugs at lower levels than conventionally used and thereby to minimize side effects. Also, by suitable manipulations of liposome structure, it may be possible to tailor a series of liposomes of graded properties to release their contents to the cornea at varying rates to enable a sustained-release application of even smaller amounts of drug, which would further reduce side effects.

Laser surgery

Laser surgery is an attractive alternative to conventional eye surgery because it is "non-invasive" and causes minimal discomfort and trauma to patients. While uses of laser energy burns to improve aqueous humor drainage are becoming prevalent, critical assessments are still required to define

optimal technical parameters, eligible patient populations, efficacy in maintaining normal IOP, and the long term benefit-risk ratios of the procedures. Pollack has recently reviewed the history and progress and technical details of laser iridotomy in treatment of angle-closure or pupillary block glaucoma.³¹ Ideally, one wishes to puncture the iris with a burn that will produce a lasting drainage channel while not thermally damaging adjacent tissues. The pulsed argon laser, which satisfies many of these technical criteria, was used to treat patients who were then monitored for periods of 1-4 years. In a series of 148 eyes so treated, a success rate of 95% was obtained in opening drainage; for technical reasons, laser iridotomy was not completed in 5% of the cases, and in these, iridectomy was successfully performed. Another series of eyes were monitored to determine rate and amount of hole-closure by pigment. All holes remaining at least 50% unblocked at six weeks following treatment remained patent. Of the 35% of initial holes showing over 50% blockage by pigment, all were readily reopened by subsequent laser treatment. For these types of glaucoma, at least, laser surgery appears to be an effective therapeutic procedure.

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Glaucoma

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SENSORY AND MOTOR DISORDERS OF VISION PROGRAM

INTRODUCTION

Over 25% of visual problems in the United States--some of the more intractable ones--result from sensory or motor disorders: strabismus, amblyopia, optic nerve degeneration, nystagmus, gaze abnormality, refractive errors. These are the concern of the NEI Sensory and Motor Disorders of Vision program. Research in this area is often multidisciplinary, involving the sciences of neuroanatomy, neurophysiology, biochemistry, biomathematics, genetics, psychophysics, behavior, bioengineering, as well as ophthalmology and optometry.

During FY 1980 the program supported about 300 grants with almost \$20 million. These grants were divided among research areas that were briefly indicated in last year's Annual Report. That report also mentioned the divisions of the program (subprograms) created by the National Advisory Eye Council in its most recent planning document.¹ That report reflected the judgment of the planning panel on sensory and motor disorders concerning research areas that needed greater emphasis in order to accelerate progress toward accomplishment of the mission of the National Eye Institute. The panel accomplished its task well; research progress is accelerating in those priority areas. Some of them need less emphasis now, for many of the problems identified are well on their way to solution.

The skills and research topics referred to in last year's Annual Report are found in each of the subprograms. However, to give a clearer picture of the scope of research in the total program a separate categorization has been used in this year's report. The major subdivisions are: Sensory Processes, Oculomotor Processes, Perceptual Processes, Visual Systems as a Whole, and Rehabilitation. Under each of these headings we will briefly discuss recent results of some representative projects.

Sensory Processes

This subprogram, like most of the others, is subdivided into three sections: structure and function, development, and disorders. Together, these comprise the largest single group of projects, containing about 58% of the number of grants and 60% of the research funds. Generally speaking, Sensory Processes encompasses the parts of the nervous system dealing with vision, starting at the optic nerve's ganglion cells and tracing the "information processing" into many parts of the brain.

Structure and function concerns the "where" and "how" of the way the information provided by light, after reception and initial processing in the retina, is further sorted, compared, and collated by other parts of the brain. Therefore, it is here projects using techniques of anatomy, physiology, and biochemistry are found if they apply to the visual nervous system.

Kaas² has found a small visual area with a complete representation of the visual field on the medial wall of the cerebral hemispheres of monkeys. The medial visual area (M) projects to area 18 and other extra striate visual areas, terminating in layer VI. The middle temporal visual area (MT) projects

to striate cortex ipsilaterally with callosal connections to the contralateral MT.

Enroth and her associates have been investigating responses of individual ganglion cells for several years. The analysis becomes more and more detailed. A test stimulus elicits fewer spikes in a ganglion cell when the cells' receptive field contains a moving stimulus even though total illumination remains constant. This suppression occurs in both X and Y cells whether they be on-center or off-center.³ Surround responses are also suppressed by a moving peripheral pattern. In a two-spot summation study of the processing of rod signals in the receptive field centers of ganglion cells, this group of investigators found that, for moderate responses, the magnitude of the response to two spots together is the algebraic sum of them when presented individually. The investigators conclude that there is a three stage processing within the receptive field center: a compressive transformation of illuminance into a neural signal, followed by a linear summation, ending with a second compressive transformation.⁴

Data collection in these experiments is onerous. Tzanakou and her associates have been developing a computer scanning-response feedback system which, when completed, will ease the strain and will present a different view of at least the trigger features of single neurons in sensory pathways. So far, pulse height and arrival time in the frog tectum, relative to stimulus presentation, can be measured, permitting the study⁵ of different cells simultaneously under identical stimulus conditions.

When the magnocellular layer of the lateral geniculate nucleus of monkeys was inactivated with lidocaine, visually driven activity of most cells in the topographically corresponding part of the colliculus was disrupted. The superficial retinotectal zone was unaffected. Inactivation of the parvocellular layer had no effect. Apparently the indirect pathway via cortex to superior colliculus is activated by the broadband system relayed through the magnocellular layer.⁶ If the frontal eye fields and superior colliculus are both removed, control of saccades is lost; disruption of one of these pathways alone produces only subtle deficits. If the two structures are simultaneously stimulated, saccades are the weighted average of individually elicited eye movements. It is probable that complete integration does not occur in either structure, but the averaging takes place in another on which each converges, possibly the pons.⁷

Mountcastle⁸ is investigating a group of cells in the inferior parietal lobule that are responsive to visual stimuli and movement. They have large receptive fields but show no information processing of visual stimuli such as orientation selectivity. They are especially sensitive to movement, both visually and behaviorally. Some, although insensitive to light per se, have visuomotor properties: tracking, fixation, convergence, saccades.

Recording from optic tract axons, Enroth and her associates⁹ have found that the response of Y cells is reduced after administration of picrotoxin, a GABA antagonist. This is not the case with X cells. Perhaps center and surround mechanisms have different pharmacologies. Strychnine, a glycine antagonist, increases a Y cell's response. In rod-driven Y cells, a GABA

antagonist decreases the center's summing area for on-center cells and increases it for off-center cells. There was no effect on cone-driven cells.

Willard¹⁰ has purified two polypeptides concentrated near neuronal plasma membranes where they interact with actin and are involved in axonal transport. One of the most rapidly transported substances in young rabbits is found in regenerating toad ganglion cells -- a growth-associated protein (GAP). Willard feels that the mammalian CNS does not produce the signal to induce a GAP or that the injured cell fails to respond in time.

In another study¹¹ of nerve growth, neonatal mouse cerebellar explants were treated with cytosine arabinoside, an inhibitor of DNA synthesis. The cortical regions developed many large neurons without lamination or other elements. Neurites were mainly Purkinje cell axons without myelinization. Antidromic stimulation inhibited spontaneous discharges. In control cultures antidromic stimulation provoked a transient rate increase.

Dow¹² is investigating the interactions between neurophysiology and behavior. A monkey pays attention to an auditory cue and must respond to red or green lights. At the same time, electrodes are placed in cortical cells specializing in foveal representation. Dow finds there is anisotropy for 45° orientations in upper layers. Striate cells' receptive fields are larger than can be predicted from minimum cone diameter or separable visual acuity. The investigator is now concentrating on a cluster of color sensitive cells in the prelunate gyrus and the lunate sulcus.

Mathematical modelling goes on in this area of the visual system. Using the limulus eye as a biological model, Knight and his associates¹³ studied the effect of an abrupt boundary on the dynamical response of a neural network, making a quantitative prediction of the way the limulus neural network responds in the vicinity of its boundary. Empirical measures of the response to moving stimuli by single optic neurons near retinal boundaries show close agreement with the model.

This is the largest unit in the NEI's sensorimotor research program. Yet, in even this brief survey it is clear how broad is our ignorance in this field. The pace of the kind of clever, detailed research mentioned above is accelerating. In time, the nervous system should be understood; the cure of many diseases await this understanding.

Development. In order to understand the normal and abnormal functions of the visual nervous system, one needs to know how it originated and how it changes over time. In this section would be found projects on genetics, embryology, post-natal growth, degeneration, and regeneration as they normally occur in the visual system and as they can be disrupted.

The visual system is delicate, easily disturbed genetically, developmentally, and experientially. Guillery and associates¹⁴ find several abnormalities apparently linked to pigmentation. Genetically distinct color phases of the mink are associated with reduced retinal pigment epithelium and abnormalities of retinofugal pathways. Abnormal geniculate innervation is distinct from that found in some Siamese cats. In the geniculate, lamina A is reduced,

C1 receives a crossed input, and there are fusions between layers receiving input from the same eye.

Shatz,¹⁵ studying the embryological development of visual connections in the cat, finds that projections from the retina to the anlage of the lateral geniculate nucleus (LGN) and superior colliculus (SC) are already present at embryonic age 32 days. By 36 days fibers have invaded their targets and lateral differences are evident. Lamination of the LGN occurs by day 55 and ten days later, at birth, segregation of retinal afferents is complete.

A more experimental way of studying prenatal growth is furnished by tissue culture. Adler¹⁶ categorizes cell types in mono-layer cultures of chick embryo retina. The growth points (neurites) are 35% monopolar, 40% bipolar, and 15% tripolar. Aqueous extract of chick embryo tectal cells accelerates growth of these neurites. Some apparent ganglion cells produce very long neurites. With this preparation, Adler is in a position to discover the pharmacology of neuron attraction.

Drager also investigates genetic visual abnormalities, both anatomically and physiologically. A common genetic abnormality in mice is retinal degeneration, starting immediately after birth so that most of the photoreceptor layer has disappeared in three weeks. The central area degenerates before the periphery and rods before cones. The longest surviving responses are of the off-center type, and loss is not affected by ambient illumination. Yet a great deal of sensitivity remains for a surprising length of time. At 18 days the extent of the representation of the visual field in the superior colliculus is normal, and although scotomata develop in 24 days, responses are still essentially normal in the periphery.¹⁷

Mammals' retinae degenerate, fish axons regenerate. Ingoglia is trying to find out why there is a species difference. After sectioning the optic nerve of goldfish, he found increased levels of uridine and adenosine compared to intact axons because they were protected from phosphorylation inside the axon. Spermadine and 45 RNA also are protected from metabolic degradation. The investigator feels these studies of chemical processes might allow us to understand why the mammalian central nervous system does not naturally regenerate.¹⁸

Much of the development of the nervous system takes place prenatally. Rakic performs the experimental manipulations on monkey fetuses, and after birth consequent to a normal gestation period, examines the developmental effects. So far, he knows there are quite different neural proliferation patterns between the inferior and superior colliculus. There are no gradients, mediolateral or rostrocaudal, in the superior colliculus. By embryonic day 54 there is some optic nerve synaptogenesis in the superior colliculus.¹⁹

One of the mysteries of the development of the nervous system remains the mechanism which leads an axon to its designated target. Lund and his associates are attacking this problem by asking what makes an axon connect on one or the other side of the brain. In the newborn rat, retinal axons from each eye are distributed across the whole area of the ipsi- and contra-lateral superior colliculus. After ten days most of the ipsilateral

projections have retracted. This retraction fails to occur if one eye is removed at birth²⁰. Rats do not show effects of dark rearing or albinism on callosal connections. Tract lesions²¹ lead to diminished collosal input, and geniculate lesions cause expansion²¹.

Another attempt toward answering the question of intercellular recognition during development is represented by the investigations of those interested in chemical attractions. Jakoi and Marchase²² have purified a filamentous protein, ligatin, from plasma membranes of embryonic chick retina. These filaments are large in both retina and ileum, resulting from their function as a baseplate for attachment of another protein, beta-N-acetylhexosaminidase, to the cell surface. Ligatin is found on the cell surface of intact retinal cells and has been visualized as a 2-dimensional lattice in electron microscopy. Marchase feels ligatin, if not an attractant, is at least an adhesive for the proper neurons²³.

Axonal transport is important in these biochemical studies of nervous system development and regeneration. Kelly finds that the proteins important in axonal and presynaptic function are similar in the collicular and geniculate pathways, except for one difference in charge modification of one protein. There are no differences in protein complement as a result of visual deprivation. Two proteins seem involved in suppressing ipsilateral nuclei. Striking differences occur between motoneurons (sciatic or femoral) and the visual nuclei²⁴.

There is a slight innate contralateral bias in the striate cortex in the distribution of ocular dominance cells. Pettigrew and his associates, by giving local perfusions of norepinehrine (NE) to kittens, were able greatly to accentuate this contralateral bias. The ratio of binocular/monocular cells was unchanged, but cells dominated by the ipsilateral eye were absent. However, this effect failed²⁵ to occur if the kittens were kept in the dark for the seven days of perfusion.

Several investigators are trying to find out what the biochemical and electrophysiological facts of the visual nervous system mean in terms of the organism's behavior. In one such study,²⁶ cortical areas 17, 18, and 19 were removed from cats. They were then trained to perform two visual discrimination tasks. Three to seven months after the cortical lesions, single neurons in the lateral suprasylvian (LS) area were recorded. Compared to recordings made immediately after the ablations, there were no changes as a concomitant of the training in numbers of active cells, their ocular dominance pattern, receptive fields, and orientation selectivity. There seems to be no reorganization of sensory coding; recovery occurs on the basis of remaining visual information.

More research is needed on normal and abnormal development of visual nervous system functioning. Although it is a very active research area now, some parts of it are barely beginning: neurochemistry and the precise details of growth, degeneration, and regeneration. Only with such information can the disorders of visual nervous system be logically attacked.

Disorders. The disorders involved include amblyopia, optic neuritis and nerve degeneration. Not all the research projects contained in this section

are investigations of the etiology, diagnosis, or treatment of one of these disorders. Projects on treatment or diagnostic tests may be a step or two from application, but studies of etiology would have to be very close to applicability to be assigned here.

Agamanolis and his associates partially deprived monkeys of vitamin B₁₂ for as long as 50 months. Some developed paralysis, but most showed some optic nerve involvement. Visual impairment was noted in all animals. Ophthalmoscopic examination disclosed optic atrophy in six of seven deficient monkeys. Degeneration was evident on postmortem in all²⁷, and there was a loss of ganglion cells in two of three animals so studied.

By injecting spinal cord myelin into young guinea pigs, Rao is able to develop chronic demyelinating optic neuritis along with encephalomyelitis. Late in the course of the disease, some animals show plaques, as in human multiple sclerosis, including gliosis and remyelination. These animals have a leakage of peroxidase tracer into the optic nerve head, and injection of leucine intravitally showed a marked reduction in grain count proximally and distally to foci of inflammation. There seems to be a deficit of axoplasmic transport in demyelinated axons.²⁸ The aim of this study is a description of optic neuritis. Human studies will be done; in the meantime, Rao has an animal model.

Sokol²⁹ has been recording the visually evoked potential (VEP) from human infants. He finds that the temporal tuning function for low spatial frequencies reaches adult levels during early infancy, but high spatial frequencies develop more slowly. In children undergoing occlusion therapy or stimulation using the Cambridge visual stimulator (CAM), VEP changes compatible with poor acuity precede subjective changes. With CAM stimulation, subjective acuity may improve temporarily, but the VEP continues to show abnormal acuity. Normal controls, subjects with ocular hypertension, and patients with glaucoma show no significant differences on Arden grating scores, but the P₁ latency of the VEP for glaucomatous eyes was larger than for the other two groups.

Levi tested suprathereshold contrast sensitivity in strabismic and anisometropic amblyopes.³⁰ At all spatial frequencies and contrast values, reaction times of amblyopic eyes were prolonged compared to the non-amblyopic eye. In the middle frequency range, contrast versus reaction time function was biphasic for normal eyes, implying that two mechanisms are used -- for high and low contrast levels. Deep amblyopia showed a monotonic curve; shallow amblyopia had the break shifted toward lower contrast. Binocular summation was absent, but binocular occlusion occurred at high contrast for amblyopes.

Agamanolis will try treating his monkeys which have early optic atrophy with radioactive B₁₂, studying optic nerve changes and site of action of B₁₂. He is also investigating possible anemia and myeloneuropathy as a consequence of chronic NO₂ administration, because the gas may interfere with B₁₂ metabolism.³¹²

Duffy³² reports the restoration of binocular input to most neurons in the visual cortex of monocularly deprived cats by administration of bicuculline, but picrotoxin, physostigmine, and strychnine were of no avail. However,

bicuculline failed to restore binocular RFs in the lateral geniculate nucleus. Bicuculline and naloxone did not increase acuity of the deprived eye in behavioral tests. Microiontophoretic injections of these substances improves results with less loss of data.

It is apparent that we have far to go in our understanding of, and treatment of, neuro-ophthalmologic disorders. Much of the problem resides in the fact that the locus of these disorders is hidden and that our diagnostic techniques do not tap the fundamental knowledge we possess, scanty as it is. However, this unit of the program is growing, and as the fundamental knowledge becomes more available and useful, clever investigators will find ways to put it to work.

Oculomotor Processes

The title of this subprogram is self-explanatory. Before, during, and after the information processing described under Sensory Processes, parts of the nervous system -- themselves intimately involved in information processing -- are less involved in the "input", or retinal information part, of the visual experience, but more involved with "output." They are concerned with what the eye muscles are doing. They direct the muscles to contract or relax, concentrate on one stimulus or another, optimizing the flow of information so that the best decisions can be made dealing with the information imparted by light when it strikes the retina. Studies of the muscles themselves are also included here. This subprogram is divided into four units: structure and function, eye movement during visual processing, development, and disorders. It accounts for about 19% of both the total grants and total grant expenditure in the Sensory and Motor Disorders of Vision Program.

Structure and function. This is the largest unit in the oculomotor subprogram. The anatomy and, to a lesser extent, the physiology of the eye muscles have been well advanced. For a complete understanding of the visual process, however, some further exploration is necessary. Far less thoroughly explained are the neuromuscular junction and the neural control of these muscles. Much more information from genetics, from embryology, and from the least understood part of an organism, its nervous system, is needed before control of strabismus and its effects is possible.

Anatomy and neurophysiology seem to go together in this field. In this way, Peterson has been investigating the reflexes and modifiable mutual interactions between eye muscles and those of the head, neck, and trunk. The medial pontomedullary reticular formation with information supplied about visual stimuli, controls direct excitation of the neck and back motoneurons, plus direct inhibition of the neck motoneurons.³³ The neck-neck reflex reduces the vestibulocollic reflex, but passive rotation of the head increases it, suggesting an input (inhibitory) from muscle spindle receptors. There is a retinotopic representation of stimuli in the superior colliculus; stimulation of the tectum results in faster eye movement when head and eyes are directed to the contralateral visual field.³⁴

These somatic phenomena help regulate the vestibulo-ocular reflex (VOR). Highstein and Reisine³⁵ find three pathways between the semicircular canals and higher centers: the median longitudinal fasciculus (MLF), the brachium conjunctivum (BC), and the ascending tract of Deiters' (ATD) which are, respectively, the inhibitory paths from anterior and posterior canals plus excitatory ones from the posterior canals to oculomotor neurons, excitatory path from anterior canal to IIIrd nucleus, and the excitatory path from ipsilateral horizontal canal to medial rectus motoneurons. The first two pathways contribute to the vertical VOR, the last to the horizontal. In effect, medial rectus motoneurons receive a head velocity signal from both the abducens internuclear neurons and from the ATD³⁶.

While varying acceleration in a Barany test with normal monkeys, Westheimer and his associates intravenously injected diazepam. They found that the drug probably has more than one site of action, for there were changes in gain and time constants of nystagmus with a reduction in directional asymmetry.³⁷ This group of investigators, interested in sensory factors, are also searching for cell types responsible for neural plasticity in a group of monkeys cerebellectomized as newborns.³⁸

Many investigators are interested in the role of the cerebellum in eye movements. For example, Robinson and his associates tested the gain (eye velocity/head velocity) of the VOR in light and dark in cats after the animals had chronically worn reversing prisms. This experience produces an adaptive reduction in gain. After bilateral lesions of the dorsal cap of the inferior olive, the adaptation was lost when tested in the dark, not in the light. The lesion had no effect on optokinetic nystagmus or optokinetic after nystagmus. The dorsal cap of the inferior olive seems necessary for plastic adaptation of the VOR, but the climbing fibers reaching the cerebellum from the inferior olive are not necessary for optokinetic movements.³⁹

The superior colliculus (SC) receives retinal input, and is much concerned with eye movement. It is one of the first and most important sensory-motor switching stations. Sparks⁴⁰ found a kind of neuron there whose spike activity was coupled to saccade output, preceding the onset of eye movement by 20 msec. If a stimulus only occasionally elicited a saccade, the spike in this neuron was invariably associated with saccade occurrence. This group of investigators also found cells in the SC that were visually responsive yet discharge prior to saccades in the absence of visual stimulation. These must be neurons reflecting eye position error and holding the information in spatial register.⁴¹ Somewhat further along the path to ocular muscles lies the pons. Here, too, can be found burst neurons which begin rapid firing a few milliseconds before the onset of change in eye position. The burst of activity lasts as long as the saccade.⁴²

Keller⁴³ has also been investigating the vestibular nucleus (VN) and the pons. In addition, some exploration for the neural locus of vergence has gone on. No neurons were encountered in the VN nor the pons that seemed related to vergence eye movements. Horizontal burst-tonic fibers in the MLF apparently arise from the abducens and carry presynaptic drive for medial rectus motoneurons. They contain information about conjugate eye position exclusively. Keller assumes, therefore, that vergence is organized rostral to the pons and projected directly to oculomotor neurons.

Another switching station between stimulus and oculomotor response is the parietal cortex. Skavenski⁴⁴ finds that 10% of the movement-related cells of the posterior parietal are coupled to eye position even in darkness. A larger proportion are related to the magnitude of the error between position and required position. Because humans can not change eye position to produce homogeneous exposure to sine wave gratings, Skavenski feels the theory of tuned spatial frequency filters in the human visual system may be due to uneven retinal exposure.

Not many grants in this sub-program deal with the ocular muscles themselves. Chiarandini finds that multiply innervated fibers of the rat inferior rectus often lack action potentials but show a graded response to transient increases of sodium and potassium conductances. Since singly- and multiply-innervated muscle fibers are difficult to distinguish,⁴⁵ intracellular recording or marking with stains and HRP is necessary.

This sub-program is tantalizing. On the one hand, we have almost global theories and near-application to the clinic. On the other, we have some obvious gaps (the fine details of neural connections, vergence, neurochemistry, the physiology and pharmacology of ocular muscles) that make for intellectual dissatisfaction and may prevent true application. However, the research is accelerating. Last year's annual report predicted major progress in this field soon; there is no reason to change that opinion.

Eye movements and perception. A small component of the Oculomotor Processes subprogram is concerned with the stimulus characteristics and cognitive factors that determine when and where eye movements will be made and the reciprocal effects of eye movements upon the information processed.

In studies at Cal Tech on the programming of saccades, Fender and his colleagues have demonstrated that computations for direction and magnitude of these ballistic eye movements are made separately. Apparently, the magnitude computation requires the results of the direction computation, but it starts before direction computation finishes. Another interesting finding is that a second saccade can start to be programmed before the first saccade is executed, but there is an approximately⁴⁶ 100 msec. refractory period following the programming of the first saccade.

That eye movements themselves can seriously affect our perception of the visual world is demonstrated by Shebilski's experiments on visuo-motor coordination. He has replicated his previous finding of significant directional pointing errors after subjects hold asymmetric eye positions (60°). With smaller deviations (30°), half his subjects showed the after effect. An interesting "real life" example of the effect was produced in experienced baseball batters. After sustained head tilt and eye elevation, the batters swung lower than normal. Felt position of the bat was ruled out as a source of the distortion, leaving altered visual perception as a possible interpretation.⁴⁷

Progress in assessing the relationship between eye movements and visual perception will depend upon the development of accurate, convenient devices for measuring the eye movements. A group of researchers at the Stanford

Research Institute has developed an increasingly widely used device called the Dual-Purkinje-Image Eyetracker that allows precise eye position monitoring. A fourth and final version of this instrument is now under development, with several significant advantages over earlier versions. Automatic alignment to the optimum position may eliminate the need for separate personnel for this purpose. The noise level of the device has been reduced from several minutes of arc peak-to-peak to less than one minute of arc. The frequency response of the system has been improved such that the total system delay may be on the order of 0.5 msec, compared to 1.5 - 2.0 msec in third-generation instruments.⁴⁸ These instruments are useful not only for precise measurement of eye movements in perceptual experiments, but their output can also be used to nullify the eye movements, thereby creating a stabilized image. Crane's group intends to couple one of their autoalignment trackers to a noncontact lens photocoagulator, giving more precise control of laser beam placement on the retina than is now possible.

Research in this area is at the boundary between the anatomical, systems-oriented approach of the studies described in the preceding section and the psychologically-oriented studies that are closer to our perceptual experience of the visual world to be described under the Perceptual Processes subprogram. The challenge will ultimately be to connect the nicely detailed circuits of the oculomotor system with what we are learning about the neural underpinnings for our various channels of sensation. However, work in this area remains mostly at the descriptive or phenomenological levels at the moment.

Oculomotor development. Despite the acknowledged importance of information on the normal development of visual structures and processes, as reflected in the upsurge of research activity in both neural and perceptual development, there is little known and little current investigation of oculomotor development.

Ornitz and his colleagues at UCLA report the successful normative measurement of vestibular nystagmus maturation in infants and children one month to eleven years of age.⁴⁹ Horizontal eye movements were recorded in the child during rotation in a modified Barany chair in complete darkness. In response to constant angular acceleration followed by constant velocity of rotation, the young infants had larger amplitude, higher velocity nystagmus beats than the older children during both primary and secondary nystagmus. Parameters describing all phases of the response reached their peak values and terminated earlier in the infant than in the older child. These results and a greater ratio of secondary to primary nystagmus in the infants were interpreted as possibly reflecting a gradual maturation of vestibular responsiveness and a more rapid development of vestibular adaptation.

In order to assess the effects of early visual deprivation on the primate oculomotor system, Sparks⁵⁰ has developed a method for behavioral testing of full visual fields and vergence, saccadic and pursuit eye movements. One monkey with early monocular lid suture developed misalignment of the deprived eye, poor fixation (even with the non-deprived eye) involving saccadic intrusions and vertical nystagmus, and no optokinetic nystagmus or response to

peripheral targets in the deprived eye.

Clearly, there are important consequences for oculomotor as well as sensory development of early visual experience. There is an obvious need for such developmental studies from every perspective applicable to the visual system as a whole. There are no obvious impediments to such research except a current lack of interest by prospective investigators.

Oculomotor disorders. By far the most common disorder of the oculomotor system is strabismus. NEI grantees have been studying the consequences of misalignment of the eyes, especially during the course of postnatal maturation, using all the anatomical, electrophysiological, biochemical and behavioral techniques that have characterized research on the basic structure and function of the visual system.

It is now clear that one main effect of strabismus is loss of binocularity. This is particularly evident in the drastic reduction in the proportion of visual cortical cells driven by both eyes.^{51,52} In cats made strabismic surgically during infancy, the percentage of binocularly activated units in area 17 increased with increasing age at surgery from 10% for surgery at 10-18 days to 50% for surgery at 60 days. By 80 days of age, surgical induction of strabismus produced a distribution no different from that found in normal adult cats.⁵¹ Thus, the critical period for loss of binocularity from strabismus ends earlier than that for monocular deprivation (MD). Interestingly, the latter critical period for MD can be extended by rearing the cats in the dark before a period of monocular exposure.⁵³

Berman and her colleagues have recently confirmed a role for the corpus callosum in the development and maintenance of cortical binocularity, since section of the posterior corpus callosum reduces the percentage of binocularly driven cells from 80+% to 37%. Additionally, they (and others) have demonstrated that surgically induced strabismus prior to eye opening, or early monocular deprivation prior to eye opening, results in abnormal callosal connections in the adult cat.⁵⁴

Sometimes raising cats in the dark and then exposing them to light results in the development of a marked convergent strabismus.⁵⁵ Removal of visual cortex in such animals can prevent the development of strabismus.⁵⁶ Thus, the complexity of postnatal visual experience and the role of various anatomical structures in the development of abnormal binocular vision are yielding to the experimental approach.

In order to assess the torsional properties of the eye before and after ocular surgery, the clinician needs a less tedious procedure for measurement than has been available so far. Nakayama⁵⁷ has devised a rapid measurement technique that only requires the examiner to line up a reticule line parallel to a prominent blood vessel on the optic disk and to depress a switch. The orientation of the ophthalmoscope is then recorded using a damped pendulum attached to a potentiometer. A microprocessor then makes the necessary calculations to test whether the rotations of a given eye are in accord with

Listing's Law.

Nystagmus is another disorder of the oculomotor system currently receiving research attention. Yee has studied patients with congenital nystagmus, demonstrating a defect in the subcortical optokinetic subsystem that produces optokinetic after-nystagmus (OKAN)⁵⁸ and that normally suppresses the vestibulo-ocular response (VOR) during simultaneous rotation of a subject within a rotating drum.

Periodic alternating nystagmus (PAN) has been modeled theoretically by a group at Johns Hopkins on the basis of eye movement recordings in three patients.⁶⁰ They found a consistent period length of four minutes in light and darkness with impaired pursuit and vestibulo-ocular responses with increased gain but short time constants. Their model of the optokinetic-vestibular system consists of an internal positive feedback loop to account for the time constant of the normal VOR and OKAN and an internal negative feedback "adaptation" loop to maintain balance between the vestibular nuclei. The model successfully accounted for the reversal phases of vestibular nystagmus (secondary nystagmus) and OKAN, and by introducing a slight gain of the positive feedback loop they induced an instability in the system such that it oscillated with a period similar to PAN. They have been able to predict the way in which the amplitude and phase of the oscillator could be changed by a vestibular stimulus and were in fact able to stop PAN temporarily. In addition, these investigators have been able to stop the PAN permanently in these patients with a GABA-ergic agent (baclofen) that possibly acts by decreasing the inappropriate gain of the feedback loop in their model. Introducing such an element into their model also halted its oscillation.⁶¹ This research is a fine example of a multidisciplinary approach (theoretical modelling, clinical neuro-ophthalmology, and neuropharmacology) that has contributed simultaneously to our understanding of normal functioning as well as the etiology and treatment of a particular disorder. In general, progress in the treatment of oculomotor disorders has been slow, but the rapidly accumulating fundamental knowledge about the structure and function of the oculomotor system is beginning to see translation to the clinical situation.

Perceptual Processes

The information received by the visual cortex is processed further by other centers in the brain and results in our subjective experience of the visual world. Objective measurement of this experience is often accomplished by behavioral techniques known as psychophysical procedures, which seek to relate various stimulus characteristics to a person's response to them. Evaluation of more sophisticated procedures that permit more precise conclusions about the neural mechanisms mediating our perceptual responses has characterized this field over the past decade. In this part of the Sensory and Motor Disorders of Vision Program there are 60 grants, representing 22% of the total, addressing the basic mechanisms, development, and disorders of visual perception.

Basic Perceptual Processes. The dominant model of visual processing is characterized by multiple independent channels tuned to different spatial frequencies and perhaps other stimulus characteristics such as orientation,

direction of movement, and location in the visual field. Threshold luminance or contrast for detection of stimuli were typically the measures of system sensitivity, and these gave rise to threshold probability summation models of visual processing. More recently, use of suprathreshold stimuli has introduced a complexity to the models, implying that there may be fundamentally different mechanisms involved in, e.g., detection versus discrimination of stimuli. Thomas⁶² employs an experimental design that permits simultaneous estimation of both detection and recognition capacities of spatially tuned mechanisms. When yes/no or rating procedures are used, an observer can discriminate between two widely differing stimuli better than he or she can detect either stimulus alone. The more traditional probability summation models cannot predict this. Because his model could predict responses to gratings but not rectangular bars, Thomas assumes that the individual mechanisms are tuned with respect to phase or position as well as spatial frequency.

The importance of suprathreshold stimulation and of target periodicity rather than just spatial frequency is also demonstrated in experiments reported by Wheeler.⁶³ He too found the probability summation model lacking in ability to account for his finding of a "foveal hole" of about 4° in diameter, outside of which visual persistence was doubled (to about 500 msec) and dependent on periodicity rather than spatial frequency. Display size is thus a more important determinant of persistence than would be concluded from threshold data alone.

Again using suprathreshold stimuli, Arend⁶⁴ has shown that for patterns containing superimposed but non-interacting frequencies, apparent contrast is determined not in a probability summation fashion but rather by the stronger of the two components. In a binocular contrast matching paradigm, Legge⁶⁵ also demonstrated that the apparent total contrast was determined by the stronger of the components.

Several investigators are now taking another look at some perceptual phenomena formerly in vogue but neglected of late: the so-called hyperacuties (e.g. vernier acuity, stereoacuity). Westheimer is interested in demonstrating the differences in physiological processing involved in "ordinary" and "hyper" acuity. He has shown that stereoscopic acuity is more susceptible to out-of-focus blur than ordinary acuity⁶⁶ and wants to investigate the relative contributions of retinal and cortical mechanisms to the total processing. Dissociation of the two kinds of acuity is further evidenced in Wheeler's observation of a total lack of correlation between vernier and Snellen acuities in 40 subjects⁶³ and the differential time course of development of stereoscopic acuity in infants compared with grating acuity (See following section on perceptual development below).

An interesting focus of recent color vision research is the presumed difference in blue versus red and green cone mechanisms of contour formation. Boynton⁶⁷ claims that chromatic contours are the result of equal but opposite excitation of the red and green cone systems on either side of the contour, while achromatic contours are the result of excitation of the R and G systems in the same direction. B cones are said to be uninvolved in contour formation. The "special" status of the blue cone mechanism is also demonstrated by the selective attenuation of its sensitivity as a function of frequency by long

wavelength light, thus distorting the modulation sensitivity function.⁶⁸

Binocular functioning is another major area of study in the Perceptual Processes subprogram. Kertesz's experiments on the cyclofusional response demonstrate the progression from basic psychophysical studies to clinical application that is the ultimate intent of the NEI program. In normal observers, a torsional disparity of 5.75° was most effective in inducing binocular torsional eye movements when stimulation was confined to an area at least 30° from the center of the visual field. When simultaneous, conflicting torsional disparities were presented to a central disk and annular surround, large peripheral stimuli induced eye movements favoring perceptual fusion in the peripheral field rather than the center.⁶⁹ Combined with an apparent learning effect that increases the effectiveness of relatively small stimuli in eliciting fusional responses following experience with much larger stimuli, these experiments formed the basis for the establishment of a Binocular Function Center at Evanston (Illinois) Hospital. Kertesz has developed a wide angle synoptoscope and a large screen, computer controlled, video fusional stimulator for the treatment of vergence insufficiencies and post-operative strabismic misalignments. He reports that 9 out of 10 patients with vergence insufficiencies experienced a significant enlargement of fusional vergence amplitudes and alleviation of symptoms such as headaches, diplopia, and dizziness. Six out of nine post-surgical strabismic patients⁷⁰ achieved significantly larger vergence amplitudes and stable ocular alignment.

The potential for clinical application of results and procedures developed to assess the normal functioning of the perceptual system is now being realized. Clinical psychophysics is a rapidly developing diagnostic field in ophthalmology and optometry, making use especially of the emerging normative data on contrast sensitivity rather than the traditional Snellen acuity. Treatment regimens based on the notions of spatially tuned psychophysical "channels" are also appearing and undergoing preliminary assessment. Further developments in our understanding of basic perceptual mechanisms can be expected to feed this new field of clinical psychophysics at an accelerated pace.

Perceptual Development. Assessment of the normal maturation of visual responsiveness and of deviations from this expected sequence has probably shown more consolidated progress during the past year than any of the other units in the Sensory and Motor Disorders of Vision program. Use of the forced choice preferential looking technique (FPL) or one of its variants has become relatively standardized across laboratories, and estimates of infants' visual acuity as a function of age are remarkable similar for different subject populations and investigators. Even cross-species comparisons now seem feasible. Boothe⁷¹ reports that visual acuity in infant monkeys, as measured by FPL, follows a similar developmental trajectory to that for human infants. As a first approximation, acuity in cycles/degree is equal to age in weeks up to 30 weeks, when the curve begins to decelerate as it approaches adult levels. Substituting months for weeks in this⁷² curve gives roughly the time course for human infant acuity development.

Extension of earlier measurements of young infant acuity to the older infant and toddler stages, otherwise notoriously difficult to assess has been

made possible by the use of operant conditioning techniques that reward the child for looking at the stimulus with higher frequency gratings. This procedure avoids the motivational problem that had plagued previous work with this age group. Variations of the operant forced choiced preferential looking technique were developed independently by Dobson at the University of Washington and Held's group at M.I.T. Dobson⁷³ has taken the important step of demonstrating the feasibility of FPL for infant visual acuity screening in the clinic setting by showing that grating acuity in 2 month old and adult subjects did not vary with luminance in the range above $-0.3 \log \text{ cd/m}^2$. Thus, screening for acuity does not require precise luminance control as long as a reasonable level of room illumination is maintained.

Using FPL, Fox⁷⁴ also has investigated the development of stereopsis in over 300 infants. He uses a modified color television to present red-green dot dynamic random element stereograms as anaglyphs capable of being seen in stereoscopic depth by observers who wear appropriate red-green filters before their eyes. The motions of a stereoscopic form moving laterally across the display serve to engage the infant's attention. Stereopsis appears in infants rather precipitously at 3 1/2 to 4 months and, unlike the gradual development of visual acuity described above, achieves high levels of performance quite quickly. Held⁷⁵ reports stereoacuity of one minute of arc or better by a mean age of 21 weeks. Fox⁷⁴ also reports that while convergence and stereoacuity develop at about the same time, neither predicts well the occurrence of the other. Infants seem equally sensitive to both crossed and uncrossed disparity and appear equally responsive to all disparity values tested in this way. They have further refined the testing method to include use of a table model video receiver to permit easy use in a clinical setting for assessing binocular functions in young infants. This should be a useful adjunct to eye position and contrast sensitivity monitoring in post-operative strabismic patients.

Teller⁷⁶ has now extended application of the FPL technique to assessment of scotopic spectral sensitivity in her young subjects. One and three-month old infants had spectral sensitivity curves that were quite similar in form to that for adults tested in the same apparatus, although their overall absolute sensitivity was depressed by 1-1.5 log units. The infants also showed a slight elevation of sensitivity in the short wavelength region of the spectrum, which Teller has suggested might be caused by a slightly more transparent lens in the very young infant. Preliminary data on premature infants in continuous illumination in a hospital nursery do not show the altered scotopic threshold that might be predicted from work with other species.

The range of perceptual capacities being assessed in infants and young children is clearly widening. With the increasing availability of behavioral procedures and the emerging electrophysiological techniques such as visually evoked potentials we should soon see a rapid expansion of our understanding of the development of visual capacity.

Perceptual disorders. The studies of the normal course of visual development described in the preceding section raise an obvious question: what happens if this normal course is disrupted? Last year's Annual Report highlighted basic neurophysiological research on consequences for development of cells located in various parts of the visual nervous system. This active area of

research continues and is now complemented by work with human infants whose visual experience has been abnormal. The main conclusion from this work so far is that many of the deficits seen following various forms of visual deprivation are reversible, giving scientific credence to our optimism for good therapeutic prognosis.

Freeman's research program at Berkeley combines basic research on kittens with clinical research on human infants. He reports that during the peak of the sensitive period to monocular deprivation, kittens reared in the dark except for brief daily exposure of first the left and then the right eye have nearly all monocular cells in cortical area 17, but no systematic imbalance in the number of units controlled by each eye.⁷⁷ This finding, combined with other work on⁷⁸ the cumulative effects of brief periods of monocular deprivation, leads Freeman⁷⁸ to conclude that patching techniques used in treatment of amblyopia are not likely to impair binocular function.

Held's⁷⁹ studies of monocular acuity following occlusion therapy for strabismic amblyopia in human infants bear out this contention. The normal rise in acuity with age was either arrested or decreased in the previously normal occluded eye, while that of the amblyopic non-occluded eye increased. The rate of change of acuity varied from a few days to several weeks, depending on the number of hours of occlusion per day. The arrested or reduced acuity of the occluded eye is reversible by either reverse occlusion or binocular exposure without any further occlusion.

Additional evidence for reversibility of deprivation effects during the sensitive period comes from studies of children with congenital cataracts.⁸⁰ This disorder provides the naturally occurring equivalent of visual deprivation in animal models. Four infants with complete bilateral cataracts at birth were assessed for visual acuity with the FPL method following surgery. All had right eye surgery at three weeks of age and left eye surgery at six weeks. In all these infants, corrected visual acuity was normal for age in the right eye and in the left equal to that first measured in the right eye. Thus, binocular visual deprivation from birth until three weeks of age produced no deleterious effect on visual acuity, and the arrested development in the eye deprived until six weeks was reversed. Both eyes then showed normal development of acuities for both eyes. Mohindra, Jacobson, and Held⁸⁰ studied one infant born with incomplete cataracts who had normal acuity development until the cataracts became complete at 10 weeks. Following removal of the cataracts at 23 weeks, acuity was at the level recorded at 10 weeks. Subsequent testing showed normal recovery of monocular acuity; again the arrested development was reversible.

Another interesting result from Freeman's work with human subjects is the finding that adults who were functionally monocular during childhood have better vernier acuity in their good eye than binocular subjects tested monocularly.⁸¹ This might be taken as evidence of an active neural process whereby additional elements or pathways become available to the functional eye as a consequence of the deprivation.

Use of the visual evoked potential as a diagnostic tool for early changes in visual functioning as a result of glaucoma has already been discussed in the sensory disorders section.²⁹ Srebo⁸² is using the same technique to look

at patients with macular degeneration. He has developed a visual stimulator that provides a homogeneous visual field modulated temporally in a pseudo-random sequence. VEP power analysis shows a selective loss in macular degeneration of the low temporal frequency range, perhaps reflecting difficulty in processing high spatial frequency inputs.

The advent of the microprocessor has revolutionized the scientist/clinician's ability to control stimuli and collect data rapidly, modifying subsequent stimulus presentations on the basis of instantly analyzed responses to previous stimuli. Ratliff⁸³ is building a micro-computer-based visual stimulator/data analyzer for both basic psychophysical research and as a diagnostic instrument, using the VEP as the principal response measure. A preliminary intriguing result of topical application of bicuculline (a GABA blocking agent) is a dramatic and completely reversible increase in the (presumed) excitatory component of the VEP. The effect is similar to a change seen in the VEPs of certain patients with Huntington's chorea.

The enormous potential for application of basic psychophysical techniques to the study of perceptual disorders has already been discussed in the section on Basic Perceptual Mechanisms. One example of such application is the demonstration of color vision defects in a variety of disorders. Young⁸⁴ has shown that the color matches of 46 retinitis pigmentosa patients are displaced toward the protanomalous side of the normal Rayleigh equation, suggesting that the optical density of the foveal cones may be reduced. Absence of the blue cone mechanism in a cortically color blind patient despite normal scotopic and photopic functions leads to the speculation that the blue cone mechanism contributes to chromaticity but not luminance channels.⁸⁵ This would seem to complement Boynton's theories,²² from a very different perspective.

Pokorny⁸⁶ also finds a shift in the Rayleigh match toward the red end of the spectrum in a variety of disorders. This color match abnormality is always accompanied by an abnormal Stiles-Crawford effect, which he interprets as evidence for disorientation of the photoreceptor layer. He has also performed a colorimetric analysis of urine-sugar tests used by diabetics. Most tests have color differences large enough for easy discrimination by normal observers, but there may be problems for those with a blue-yellow defect secondary to diabetes or with congenital red-green defects seen in 10% of the male population.

This overview gives an indication of the developments in clinical psychophysics in the past year. As mentioned earlier, it is clear that this field will soon be one of the most productive in visual science.

Visual System as a Whole

It is apparent that this category contains projects that do not fit nicely elsewhere--ones that overlap too many other categories or work on problems affecting all of them. The visual system category accounts for 3% of the number of grants and total grant funds in the Sensory and Motor Disorders of Vision Program. Although this subprogram has the same three units, structure-function, development, and disorders as previously mentioned, here we will discuss these few grants together.

In⁸⁷ a painstaking anatomical study of a series of staged human embryos, Pearson describes the intricate details of intrauterine development of all parts of the visual apparatus. In two months, after a lens placode is formed with its closed pit, grooves form first above and then below the eye and then grow to close over until late in pregnancy.

In another project involved in eyelid function, Scott⁸⁸ has developed a strain gage to measure lid position, direction of movement, and force of movement. With this device, requiring no anesthetic and tolerated well by both adults and children, information is being collected on normal subjects dealing with force and velocity of eye and lid movement, comparing blinks and saccades. These normal data will be contrasted with those collected from patients with ptosis, myasthenia, and ophthalmoplegia.

Miles is investigating chronobiological dysfunction (including sleep disorders) in the blind. Although the subjects are still being recruited, data are being collected on circadian rhythm: temperature fluctuations, diurnal variations in hormones and metabolic functions, and other rhythmic phenomena in order to discover the extent and severity of these distortions of normal rhythm resulting from lack of visual information.⁸⁹

Graves' disease often has ophthalmic concomitants varying from mild to severe and affecting almost any part of the visual system: lid, pupil, extra-ocular muscles, optic nerve, and the globe as a whole in exophthalmos.⁹⁰ Savino is investigating the immunologic aspects of Graves' ophthalmopathy. Since thymosin suppresses rosette formation in vitro and patients with the most favorable therapeutic response to corticosteroids also show increased T-lymphocyte levels, this class of disorder may be viewed as a defect in T-cell maturation caused by inadequate thymosin function.

Wiesel and Raviola are continuing their studies of experimental myopia. The increase in axial length of a lid-sutured eye probably cannot alone account for its high refractive error, and it does not develop in lid-sutured eyes of monkeys raised in the dark. Also, axial length increases after corneal opacification;⁹¹ some inequality of transmission of light in the entire optical system probably causes this myopia. Believing excessive accommodation may account for it, these investigators daily instilled atropine into the closed eye of⁹² two monkey species; the drug prevented axial myopia in one, not the other.

McKanna and Casagrande found elongation of the globe insufficient to account for the degree of myopia encountered in lid-sutured eyes of another primate, the tree shrew. They also favor an excessive accommodation hypothesis but are investigating all refractive elements in rabbits, rats, kittens, and chickens as well as tree shrews. Deprived eyes develop zonular dysplasia, their lenses are smaller, the pupils do not equal the non-deprived eyes, and the axis becomes elongated. The investigators believe that all these facts support their hypothesis that deprivation myopia comes about from nonconsequential accommodation, an effect of emmetropization via intersecting feedback loops.

Although this is, perforce, a heterogeneous subprogram, it contains some unique grants. It involves some important clinical problems; myopia, for

example. Myopia is a socio-economic problem of very large scale. More research effort in myopia is obviously needed; this subprogram is likely to grow.

Rehabilitation

This section of the Sensory and Motor Disorders of Vision program is currently quite small, despite the emphasis accorded rehabilitation research in Vision Research--A National Plan: 1978-1982.⁹⁴ The program supports biomedical and behavioral research related to rehabilitation of blind persons and those with low vision. The Institute has made a concerted effort in the last year to increase this activity, particularly in low vision research. To that end, a small workshop in February 1980 identified a number of promising research directions aimed at improved characterization of the residual visual capacities of patients with low vision and maximizing their use of these capacities. The NEI has also taken the lead in establishing an interagency work group to coordinate the federal government's support of research on low vision.

Actual progress from rehabilitation research is exemplified in the results of two NEI grants. Legge,⁹⁵ of the University of Minnesota, has developed a low vision reading aid based on discrete sampling of a printed text presented in the form of a matrix display. He has found that low vision is not equivalent to low resolution normal vision, for even when letter size and sampling density are within the resolving power of the low vision observer, his reading rate is depressed relative to that of the normal observer reading the same text. This difference may be due to differences in the shapes of their contrast sensitivity functions, differential effects of visual masking, or differential use of information in broadband, low spatial frequency channels. This work thus gives clues to potentially fruitful avenues of psychophysical research with low vision patients.

The second project involves the development of a mobility aid for the blind based on maximizing use of the blind person's ability to use cues in other sense modalities for information about his own direction of movement. Rowell⁹⁶ has found that magnitude of tracking error cued by loudness is more effective than constant loudness with interaural intensity difference cueing directional error. This is of particular significance because an existing mobility device uses the latter, less effective, form of display.

Clearly, more high quality, imaginative research is needed to solve the problems of effective functioning world by those with severely impaired vision. There are definite opportunities for significant contributions to this field now; it will be a challenge for the NEI Sensory and Motor Disorders of Vision program to generate interest in this area among scientists and clinicians who possess the skills necessary to carry out this work.

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OFFICE OF BIOMETRY AND EPIDEMIOLOGY

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REPORT OF THE CHIEF, OFFICE OF BIOMETRY AND EPIDEMIOLOGY
Fred Ederer

The Office of Biometry and Epidemiology has three main functions: research, education, and consultation.

Research is the dominant function. It is the Office's mission to plan, develop, and carry out studies on human populations concerned with the causation, prevention, and treatment of eye disease and vision disorders, with emphasis on the major causes of blindness. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures.

Education: The Office carries out a program of education in biometric and epidemiologic principles and methods for the vision research community. This consists of courses, workshops, a pre- and post-residency fellowship program for ophthalmologists, publications, and consultation and collaboration on research.

Consultation: The Office provides biometric and epidemiologic assistance to National Eye Institute intramural and extramural staff and to vision research workers elsewhere. The assistance ranges from referral to appropriate consultants to collaboration as coinvestigator.

Research

Clinical Trials. Three clinical trials on the treatment of diabetic retinopathy are in progress. These are the Diabetic Retinopathy Study (DRS), the Early Treatment Diabetic Retinopathy Study (ETDRS), and the Diabetic Retinopathy Vitrectomy Study (DRVS).

The oldest of these is the DRS, which demonstrated effectiveness of photocoagulation in reducing the incidence of diabetic blindness. Recently, two more DRS publications have appeared, one summarizing follow-up to five years after randomization, the other focusing on complications of photocoagulation in different patient groups. A monograph describing the characteristics of the patients at baseline and providing a detailed description of study methods has been submitted for publication. Other analyses are being carried out, and archival tapes are being prepared to be made available to investigators who wish to use this research resource.

The ETDRS was designed to provide a better understanding of the optimum time to use photocoagulation in the course of diabetic retinopathy. Patients with macular edema and with preproliferative and proliferative retinopathy are included. Three forms of photocoagulation treatment will be assessed, ranging from a restricted focal treatment to a full scatter. In addition,

half the patients will be randomized to a daily administration of aspirin to test the effect of this drug on the incidence of microvascular complications. Of additional interest is whether aspirin reduces macrovascular complications in the diabetic patient. The study will also provide the opportunity to investigate the factors that are associated with the progression of disease. Over one hundred patients have been admitted to the study, with recruiting likely to last for at least two more years. The DRVS has succeeded in recruiting over 400 patients whose vision has been reduced by hemorrhage into the vitreous (group H) and over 100 patients who still have useful vision but with serious risk of complications leading to retinal detachment (group NR). Half of these patient eyes have been randomized to early vitrectomy, and half to vitrectomy one year later, if still indicated (in group H), or to "traditional" care in group NR.

Epidemiologic Research. Little is known about the frequency of eye disease and visual impairment in the United States, how this frequency varies according to various demographic and social factors, or how it varies over time. Such information is fundamental to the formulation and testing of hypotheses in the epidemiologic research of vision disorders. Previous efforts to collect such information, including NEI's Framingham Eye Study, the National Center for Health Statistics' (NCHS) Health and Nutrition Examination Survey, and NIH's Model Reporting Area for Blindness Statistics, have been limited in scope or assurance of quality.

During the past year, a project team consisting of OBE Staff and several consultants has been preparing a plan for a pilot study for the Visual Acuity Impairment Survey (VAIS), a two-stage national survey of visual impairment in the United States. The primary objective of the study is to determine the nationwide prevalence of visual impairment, by cause. A secondary objective is to conduct case-control studies to investigate etiologic hypotheses, using cases of visual impairment from a specific cause and, as controls, a random sample of survey participants not visually impaired from that cause. In the first stage, visual acuity examinations would be carried out in a sample of households as part of the NCHS Health Interview Survey, a continuing survey of a probability sample of 42,000 households (120,000 individuals) per year. In the second stage, all those found to be visually impaired in the first stage plus a sample of those not found to be visually impaired would be given a detailed ophthalmological examination. A Request for Proposals was issued in August for two or three clinical centers, a data center, and a reading center. The pilot study is planned for fiscal year 1981; the study should proceed at full scale in fiscal year 1983.

A baseline monograph along with a summary paper was published on the Framingham Eye Study in the Survey of Ophthalmology. Accompanying the paper was an editorial that was laudatory. The monograph contains detailed clinical and epidemiologic findings and details of study methods. We anticipate that the monograph will serve as a research resource and teaching tool for ophthalmic-epidemiologic researchers.

Dr. Roy Milton collaborated with Mr. Harold Kahn, a consultant in epidemiology, in two studies using Framingham Eye Study data: revised estimates of the prevalence of glaucoma and diabetic retinopathy, and alternate definitions

of glaucoma and resultant prevalence estimates and associations with Framingham Heart Study variables. The latter study found a previously unreported association between glaucoma and alcohol use.

Dr. Milton's work with a team of consultants, to develop a plan for a nationwide prevalence survey of nutritional eye disease in preschool children in El Salvador, was ended due to political instability in El Salvador. Continuing efforts in international collaboration in research on nutritional eye disease included a visit by Drs. Guillermo Arroyave and Luis Mejia from Guatemala, and a visit by Dr. Vinodini Reddy from the National Institute of Nutrition, Hyderabad, India. Together with Dr. Seigel and Dr. Reddy, Dr. Milton prepared a research protocol for Evaluation of a Massive Dose Vitamin A Program in the Control of Nutritional Blindness in Children, for possible collaborative implementation in Hyderabad. A paper by Dr. Milton on evaluation of the efficacy of programs for the control of xerophthalmia has been prepared for presentation at the October 1980 International Vitamin A Consultative Group (IVACG) meeting in Jakarta, Indonesia.

Mr. Podgor has continued to work with Dr. Robert Frank, Kresge Eye Institute, Wayne State University, in an extension of a study of retinal vascular changes in juvenile onset diabetes of short duration. Mr. Podgor is also collaborating with Dr. Muriel Kaiser, Clinical Branch, NEI, on studies of low ocular rigidity measurements in patients with osteogenesis imperfecta and of pupillography in patients with pigment dispersion syndrome. Results on low ocular rigidity were presented at the 1980 meeting of the Association for Research in Vision and Ophthalmology. Mr. Podgor and Mr. Ederer collaborated with Dr. M. Cristina Leske, State University of New York at Stony Brook, in a study of the usefulness of Framingham Eye Study data in planning glaucoma screening surveys.

Little is known about the etiology of senile macular degeneration, a major cause of blindness in the United States, and in an attempt to test various etiologic hypotheses about the disease, a case-control study has been conducted by OBE staff collaborating with epidemiology and ophthalmology staff at Johns Hopkins University. All interviews have now been completed, and clinical data both abstracted and coded, on over 200 each of cases and controls. Editing of the data is now under way, soon to be followed by analysis.

The debate continues whether enucleation in eyes with uveal melanoma increases the risk of metastasis and death. This issue was singled out as an important one in an annual review of research in ophthalmology published in the American Medical Association Journal. A paper by Drs. Seigel and Ferris on patterns of mortality was cited for its dominant role in this debate.

Several OBE staff members collaborated with staff of the Department of Ophthalmology, University of Wisconsin, in an investigation into the survivorship of people with diabetic retinopathy. The study confirmed a previously found association between severity of retinopathy and survival and was able to quantify the association more precisely than was possible previously. The paper has now been published.

Mr. Ederer and Mrs. Hiller collaborated with Dr. Hugh Taylor of Johns Hopkins University in demonstrating a marked excess in the prevalence of senile

cataract in diabetics under age 65, both in the Framingham Eye Study data and the Health and Nutrition Examination Survey data. The paper has been accepted for publication.

Dr. Sperduto, along with Mrs. Hiller and Dr. Seigel, analyzed data from the Framingham Eye Study which showed that nuclear lens changes have a negative association with senile macular changes. They suggested that the nuclear changes might be protecting the macula from radiation-induced pathology.

Insofar as lens changes taken as a group are concerned, though both lens and macular changes increase in prevalence with age, the two conditions are not associated, suggesting independent etiologic mechanisms. These results were analyzed by Drs. Sperduto and Seigel and were reported this year.

Education and Consultation

Mr. Ederer and Dr. Ferris participated in the first of a series of courses on epidemiologic and biostatistical approaches to clinical vision research. Along with university colleagues, they presented a two-day course at Dartmouth College to clinical investigators. Attendance was excellent, and written evaluation indicated that the course material was quite appropriate. Similar courses will be offered in other parts of the country.

Mr. Ederer participated as a faculty member in a workshop in epidemiologic methods, sponsored by the Society for Epidemiologic Research.

Dr. Ferris has been very active in giving talks to various professional groups on diabetic retinopathy and in describing application of the DRS to clinical practice.

Dr. Seigel lectured at a workshop on clinical trials before the annual American Academy of Optometry meeting. In addition, he has written a chapter on clinical trial methods for a new book on ophthalmology.

Dr. Milton participated in a World Health Organization Task Force on Methods of Assessment of Avoidable Blindness, and in a review of a Society for Epidemiology and Voluntary Assistance (SEVA) Foundation proposal for a Nepal Blindness Survey. This meeting took place in San Francisco in 1979. He also represented the NEI at the January 1980 meeting of the Vision Screening Advisory Committee of the National Society to Prevent Blindness.

Both Drs. Seigel and Ferris were frequent consultants to the National Institute of Arthritis, Metabolism, and Digestive Diseases, particularly in planning clinical trials in diabetes control. They also served on workshops preparing a report on the current status and future directions in diabetes research.

Mr. Ederer serves as Epidemiology Editor for the Survey of Ophthalmology and is on the Editorial Board of the American Journal of Ophthalmology. Dr. Seigel is an Associate Editor for the American Journal of Epidemiology (his appointment has just been extended for an additional three years). Mr. Ederer is a member of the Board of Directors of the newly founded Society for Clinical Trials.

Drs. Milton and Seigel were particularly active in assisting the NEI Director in developing collaborative research programs with other countries. Through cooperative agreements with the Soviet Union and the P.L. 480 program, research protocols were developed to evaluate the drug ENKAD in the treatment of retinitis pigmentosa, and on the etiology and treatment of eye disease related to severe malnutrition.

Dr. Milton and Mr. Podgor provided statistical consultation and assistance in computer applications to members of the Clinical Branch, NEI.

The National Eye Institute is represented through Dr. Milton on the NIH Advisory Committee for Computer Usage, Dr. Seigel on the NIH Clinical Trials Committee, and Mr. Ederer on the NIH Epidemiology Committee.

Publications

Office of Biometry and Epidemiology

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17. The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: Relationship of adverse treatment effects to retinopathy severity. Diabetic Retinopathy Study (DRS) Report Number Five. Chapter in Modern Problems in Ophthalmology (Proceedings of the 1979 Meeting of Club Jules Gonin) (in press).

* Dr. Seigel served as a member of the Epidemiology and Genetics Workgroup.

** Dr. Ferris served as a member of the Ocular Complications Workgroup.

CONTRACT NARRATIVE

Fifteen Clinical Centers plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland, and a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Study (DRS)

Principal Investigator: Matthew D. Davis, M.D. (Study Chairman)

Current Fund Allocation: (No new funds allocated in this fiscal year.)

Objectives: The Diabetic Retinopathy Study (DRS) is a multicenter clinical trial to evaluate the efficacy of photocoagulation, (argon laser and xenon arc) in the treatment of proliferative diabetic retinopathy. This randomized, controlled study involves 1,758 patients enrolled at 15 medical centers.

Major findings: Photocoagulation with either argon laser or xenon arc, as used in the study, is effective in reducing the risk of severe visual loss and in inhibiting the progression of retinopathy. These effects were apparent in all stages of diabetic retinopathy studies: proliferative, severe non-proliferative, and background. Also found were some deleterious effects of treatment, namely, small losses of visual acuity and constriction of the peripheral visual field.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy, uncommon only a few decades ago, is now a leading cause of blindness and visual disability in the United States. There is a critical need to find and evaluate scientifically treatments which will reduce the risk of blindness or visual impairment from the ocular complications of diabetes. Although photocoagulation is widely used as a treatment, adequate evidence of its efficacy is not based on carefully documented research findings.

Proposed Course: Follow-up of all surviving DRS patients terminated in May 31, 1979. Since this time the data have been edited in preparation for many analyses. The baseline monograph has been completed and submitted for publication. A paper on the modification of the Airlie House Classification and its use in classification of diabetic retinopathy in the DRS has been prepared. An additional paper on the reproducibility of this grading system is now ready for publication. A preliminary report on long term follow-up of DRS patients and a report on adverse treatment effects in eyes with severe retinopathy have been published. Numerous other papers are in preparation including: assessment of risk factors for severe visual loss and death, a final paper on the treatment effect utilizing all follow-up data, mortality papers, the effect of treatment on macular edema, and many others.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publications:

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: A short report of long range results. Diabetic Retinopathy Study (DRS) Report Number Four. Excerpta Medica (in press).

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: Relationship of adverse treatment effects to retinopathy severity. Diabetic Retinopathy Study (DRS) Report Number Five. Chapter in Modern Problems in Ophthalmology (Proceedings of the 1979 meeting of Club Jules Gonin). S. Karger AG, Basel (in press).

CONTRACT NARRATIVE

Thirteen Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland; a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Vitrectomy Study (DRVS)

Principal Investigator: Matthew D. Davis, M.D. (Study Chairman)

Current Fund Allocation: \$1.3 million for the period June 26, 1980 through June 25, 1981.

Objectives: The DRVS is a multicenter clinical trial to:

- a. Evaluate vitrectomy performed in the first six months after severe vitreous hemorrhage secondary to diabetic retinopathy as compared to the more usual practice of waiting twelve months after vitreous hemorrhage to remove the vitreous.
- b. Evaluate vitrectomy in eyes with good vision but with severe proliferative retinopathy and poor prognosis before vision is lost through hemorrhage or retinal detachment.
- c. Study the natural history of progression of retinopathy.

Major Findings: As of June 1980, a total of 405 eyes with severe hemorrhage had been randomized to early or deferred vitrectomy. Follow-up continued on the 742 eyes recruited in the natural history study, where recruiting had stopped. A total of 89 eyes have been randomized in group NR.

Meeting the recruiting goals of the study remains the most difficult aspect of the study. Four clinics, with the least success, were asked to discontinue recruiting efforts and to continue follow-up of patients until June 1981. These clinics are located in New York City, Philadelphia, Chicago, and San Francisco. At the same time, a Request for Proposals (RFP) was issued for new clinics to join the study. Some of these are expected to join the study in the fall of 1980.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three (macular degeneration, glaucoma, cataract) in that it affects a younger population. Vitrectomy has the theoretical potential of removing the "scaffolding" on which abnormal new vessels can develop, fibrous tissue can form, and retinal detachment can occur. It is important to determine when such intervention is most likely to deter this process, and reduce the incidence of loss of vision. This presents an ideal opportunity for the National Eye Institute to mobilize scientific talents to answer a significant medical question.

Proposed Course: At this date 9 clinics continue to recruit and enroll new patients to the study. Thirteen clinics continue with follow-up. Recruiting is likely to continue until the projected sample size is obtained, unless the Data Monitoring Committee requests a change of protocol.

An editorial is in press in the Archives of Ophthalmology describing the study and encouraging referrals. Plans are now being formulated for publication of two year follow-up data in the natural history study, the goal being to produce a manuscript by the end of 1980.

Administratively, the study needs to integrate rapidly any new clinics that are added as a result of the RFP.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publication: Kupfer C: A new patient group in the Diabetic Retinopathy Vitrectomy Study. (Editorial) Arch Ophthalmol (in press).

CONTRACT NARRATIVE

Nineteen Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland; a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin; a Central Laboratory at the Center for Disease Control, Atlanta, Georgia; and an Electrocardiogram Reading Center at the University of Minnesota, Minneapolis, Minnesota

Title: Early Treatment Diabetic Retinopathy Study (ETDRS)

Principal Investigator: Dr. Lloyd Aiello (Chairman)

Current Fund Allocation: \$4,078,000 for the period September 30, 1980, through September 29, 1981.

Objectives: The Early Treatment Diabetic Retinopathy Study (ETDRS) is a multicenter, randomized clinical trial, the main goals of which are:

- a. To determine whether treatment of early stages of proliferative and nonproliferative diabetic retinopathy with or without macular edema by aspirin and/or prompt photocoagulation is effective in decreasing the rate of development of known retinopathy risk factors and/or the development of severe visual loss when compared to placebo or deferred photocoagulation.
- b. To determine the optimum time to initiate photocoagulation treatment in diabetic retinopathy.
- c. To monitor closely the effects of diabetes mellitus and/or of photocoagulation on visual function.
- d. To develop natural history data that can be used to develop or confirm etiologic hypotheses or identify risk factors in diabetic retinopathy.

Major Findings: All clinics were fully certified by the Study Monitoring Committee to begin recruitment by the spring of 1980. As of September 1980 approximately 234 patients had completed their qualifying visit examinations and of these, 128 patients had been treated. Monthly assessment of each clinic's progress has been conducted since April by the Clinic Monitoring Committee.

Significance to Biomedical Research and the Program of the Institute: The Institute regards fostering careful evaluation of new and widely used ophthalmic treatments as an essential element in its mission. This study represents an extension of the Institute's interest in developing the best possible program to care for the patient with diabetes.

Proposed Course: Follow-up of all ETDRS patients is planned for five to seven years, and monitoring of accumulated data will be performed at three-month intervals. The first Data Monitoring Committee meeting will be held in December of 1980.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy
and Other Vascular and Circulatory Abnormalities

Publications: None

OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1979 - September 30, 1980

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING
Julian M. Morris

Program Planning and Evaluation

During the past fiscal year, the Office's program planning and evaluation staff was occupied primarily with the development of the National Advisory Eye Council's comprehensive NEI program evaluation and national plan for the fiscal years 1983-1987. During the first quarter of the year, the planning staff worked with the Council's Program Planning Subcommittee in organizing five panels of experts to assess research needs and opportunities in each of the Institute's five programs. The Council charged each panel with:

- Reviewing in detail the report for its area of responsibility that appeared in the 1978-1982 plan and assessing that report's relevancy to the current state of research and the NEI's program in the field;
- Redefining and ranking program objectives;
- Gathering and Reviewing available data on the social and economic impact of the group of diseases with which the panel is concerned;
- Systematically reviewing and assessing current support of research by NEI and other public and private agencies in the panel's area of concern;
- Assessing and documenting recent research accomplishments in the field;
- Delineating major research needs and opportunities as well as obstacles to future progress in the field;
- Identifying and ranking according to priority the most promising research and other approaches to solving these problems and fulfilling program objectives;
- Estimating resources that will be needed to carry out these recommendations during the years 1983 to 1987;
- Identifying cross-program topics and issues and making other recommendations to the NAEC Program Planning Subcommittee to be considered for inclusion in the Subcommittee's overview report (Volume One); and
- Preparing a final report for submission to the Council's Subcommittee.

The planning and evaluation staff developed background materials for the use of Subcommittee and panels in carrying out these responsibilities. These materials include:

- A series of papers orienting consultants to the NAEC/NEI program evaluation and planning system;
- A detailed charge to the program panels and consultants who are assisting the Subcommittee;
- Data on vision research projects supported by the NEI and other organizations in the United States;
- "Tracking" data that compare the recommendations made in the most recent Council plan in 1977 with subsequent NEI funding in these areas;
- Guidelines for preparing the new plan;
- A timetable for the plan's production.

Office planning and evaluation staff presented these materials to the Subcommittee and program planning panels at a series of orientation meetings and helped panel chairmen plan, organize, and conduct eight subsequent panel meetings held during the second and third quarters. (These meetings were supported by HHS set-aside evaluation funds.) During the fourth quarter, the staff coordinated presentations to the Subcommittee of outlines of the five panel reports.

The tracking data, mentioned above, are an important tool for assessing the extent to which the 1978-1982 vision research plan has been fulfilled. The data indicate which research areas identified as high priority in the plan have received NEI support, and how much. They also help identify areas of research that may now be adequately funded or, conversely, underfunded, and thereby aid the Council and its consultants in identifying gaps in NEI support and in recommending updated priorities.

The system is also being used to track incoming grant applications, indicating how well applications submitted in priority areas fare in the NIH/NEI review process. This analysis will continue in the future as an ongoing part of the NEI's program evaluation and planning activities.

As a separate program evaluation project, the Office also used set-aside evaluation funds to support a contract for a detailed assessment of the NEI's planning and evaluation activities over the past decade. This project, to be completed early in FY 1981, is aimed first at documenting the history and background of the NEI's comprehensive evaluation and planning activities. The report will assemble for the first time in one document information on the rationale for NEI program planning and evaluation, the methodology employed, the roles of the various participants, and the outcome. The report will conclude with an assessment of this system and include recommendations for future evaluations and plans.

The planning and evaluation staff also prepared the NEI portions of the NIH Evaluation Plan for FY 1981 and the NIH Research Plan, FY 1982-1984. As in previous years, Office staff served on other Institutes' ad hoc evaluation and planning contract review committees. For example, Mr. Morris served on a panel to review proposals for an evaluation of the National Institute of Dental Research's Craniofacial Anomalies Program and is serving on the National Institute of Child Health and Human Development's editorial advisory committee for development of that Institute's national plan. Mr. Gillen assisted the National Institute on Aging by reviewing proposals for technical and logistical contractor support for the upcoming White House Conference on Aging. The planning and evaluation staff also provided editorial services for other offices in the NEI, drafted the Director's Opening Statement for Congressional appropriations hearings, and responded to special requests by members of the National Advisory Eye Council for planning and evaluation data. During FY 1980, Mr. Gillen served as Chairman of the NEI's Equal Employment Opportunity Committee and was the NEI Representative to the NIH Recreation and Welfare Association.

The Office also wrote, contributed to, commented upon, or coordinated NEI's contributions to the following reports:

- HHS Health Research Plan Initiatives;
- Emerging Health Technologies;
- Second Biennial Report to Congress on Research Activities of Relevance to the Clean Air Act;
- Federal Inventory of Population Research;
- NIH Research Strategy Plan for FY 1982-84;
- Congressional Report on HHS Drug Abuse Activities;
- 1982 PHS Program Plan;
- Miscellaneous reports including NEI funding for clinical research, for alcohol-related health hazards, and several reports related to NEI disease prevention activities.

The Office also coordinated the preparation and supervised publication of this NEI Annual Report.

Scientific Reporting

The Information Office greatly expanded its press relations activities this year in a concerted effort to establish the National Eye Institute in the media's view as a source of accurate, up-to-date information on eye disease and vision research. The long-range goals are a better informed public and improved health care--made possible by increasing the quantity and improving the quality of information on these subjects which is readily available to laymen and health care professionals.

Expansion of the National Eye Institute's information program was made possible by streamlining the handling of public inquiries, thereby reducing the amount of staff time spent on this activity, and by adding two very experienced writers to the staff. When Charlotte Armstrong left the NEI to take a job in the Clinical Center Information Office, Mary Lynn Hendrix joined the staff to serve as a science writer and to plan and develop information materials for the NEI's expanding clinical trials program. The second new staff member is Maureen Mylander, who is responsible for planning and developing public information materials, including brochures and exhibits.

Scientific Communications

At the end of FY 1979, the Office helped to plan, organize, and run the NEI's first consensus development conference, the subject of which was intraocular lens (IOL) implantation. The conference provided a forum for discussion of the safety and efficacy of IOLs by ophthalmologists, vision researchers, and the general public. The broad guidelines formulated by conference participants were incorporated into a booklet which was published during the first quarter of FY 1980 and then widely distributed to both the general and medical press. Nationwide press coverage, including articles in medical journals and medical news publications, resulted, and many clinicians called the NEI for additional information.

Another high priority this year has been the planning of a major information campaign on diabetic retinopathy and its treatment. The Diabetic Retinopathy Study results, showing that photocoagulation can halve the risk of severe visual loss in people with advanced disease, are being incorporated into a variety of informational materials for both health care professionals and the general public (see Consumer Education below). In addition, brochures and an exhibit on the Early Treatment Diabetic Retinopathy Study are being prepared to explain the objectives of this study, describe which patients are eligible, and ask physicians to refer patients. The exhibit will be displayed at the American Academy of Ophthalmology meeting in Chicago in November 1980 and at other national and local medical meetings.

The Office also provided assistance with patient recruitment for other NEI-supported clinical trials. A letter requesting patient referrals was sent to all ophthalmologists in the country. An exhibit on 13 clinical trials supported by the Institute was produced and displayed at the American Academy of Ophthalmology meeting in November 1979. In addition, the research protocol for each trial was summarized in a new booklet for physicians,

Clinical Trials in Vision Research, which was printed to accompany the exhibit and distributed at the meeting.

The Office was consulted about development of a press relations program for the Association for Research in Vision and Ophthalmology. Information was provided about planning for and running a full-scale pressroom operation at the society's annual meetings.

A status report on marihuana research was prepared for publication in the Journal of the American Medical Association's "From the NIH" column. Widespread newspaper publicity about marihuana as a possible treatment for glaucoma had generated considerable concern about this subject among ophthalmologists and optometrists, as well as the general public. Other notices were sent to professional journals to encourage the submission of research grant applications in certain areas and to announce changes in NEI grant policies.

The Information Office also assisted Dr. Kupfer in his preparation of speeches for presentation at the Washington Academy of Medicine and the Helen Keller Centennial Congress and of an editorial marking the tenth anniversary of the NEI, which was published in the American Journal of Ophthalmology.

Consumer Education

In FY 1980, plans were laid for a major information campaign aimed at health care professionals (see Scientific Communications above) and at the nation's 10 million diabetics. The long-range goal of the campaign is to reduce the incidence of severe visual impairment from diabetic retinopathy by: increasing public awareness of the ocular complications of diabetes, emphasizing that an effective treatment (photocoagulation) is available, and urging people who have had diabetes for five years to have their eyes checked. Voluntary organizations and professional societies will be contacted to enlist their cooperation and assistance in implementing the campaign. A key document in the campaign, a brochure entitled Diabetes and Your Eyes, has been drafted. In addition to the brochure, public service announcements will be prepared in cooperation with the Audiovisual Branch of NIH, and a press conference and news releases planned to announce the campaign.

In anticipation of widespread publicity about radial keratotomy, a surgical procedure to correct myopia, the Information Office prepared a fact sheet on refractive keratoplasty to put this operation in perspective, and the information was distributed upon request. When the National Advisory Eye Council adopted a resolution expressing grave concern about the potential widespread adoption of radial keratotomy without further research, the Information Office issued a news release explaining the resolution. The Council's warning reached millions of Americans when it was published in over 200 newspapers and broadcast on radio and television stations.

Inquiries from the Congress and general public about marihuana as a possible treatment for glaucoma provided another opportunity for the Office to disseminate information on the eye and explain the importance of research

in evaluating new treatments for eye disorders. Widespread newspaper publicity apparently created the impression that smoking marihuana had been proven effective in treating glaucoma, and perhaps more effective than conventional medication. Since no definitive clinical studies have been completed, this assumption was misleading and could have resulted in serious ocular damage, systemic side effects, and legal problems for the patient with glaucoma. In response to the inquiries, thousands of fact sheets on glaucoma and marihuana were distributed by the NEI directly, and by other professional societies and voluntary organizations. Tens of thousands of NEI fact sheets on other eye disorders have also been distributed jointly by the Office and by other groups.

This year an analysis was conducted of all public inquiries received by the Office in an effort to determine whether new consumer information materials are needed, and on what eye disorders. In addition, the need for more information on diabetic retinopathy and its treatment was assessed. As a result of these studies, consumer education and knowledge transfer activities for the Institute are undergoing expansion. In particular, brochures on diabetic retinopathy, cataract, and macular degeneration were indicated.

Public Inquiries

The Information Office's system for handling public inquiries was streamlined this year in an effort to expedite handling of letters and telephone calls and reduce the amount of staff time spent on this activity. The Office continues to provide complete and accurate information, but is often able to reduce drastically both the response time and paperwork. This has been accomplished by answering many letters by telephone, thereby eliminating the need for specially drafted letters. Following the telephone call, printed materials may be sent, but no covering letter is required. Response time has also been reduced by continuing to develop standardized responses which can be sent out without delay when similar questions are asked, and by preparing more fact sheets.

Press Relations

The Information Office greatly increased its press relations activities this year, most spectacularly with a news release explaining the NAEC's resolution on radial keratotomy (see Consumer Education above). Following publication of the release in well over 200 newspapers and magazines, a number of the reporters have called back to ask for information on other subjects or to ask for leads to stories that might be of interest to their readers.

The consensus development conference on intraocular lenses served a similar purpose and provided another opportunity to educate the general public about an eye problem (cataract) while disseminating information on a controversial--and newsworthy--subject (intraocular lenses). Articles summarizing the consensus conference recommendations appeared in over 100 newspapers and magazines, as well as in medical journals and medical news publications.

A treatment for retinitis pigmentosa developed in the Soviet Union continued to stimulate widespread newspaper coverage this year, as did marihuana as a possible treatment for glaucoma. (see Scientific Communications and Consumer Education above). The Information Office fielded questions on these subjects by reporters from newspapers across the nation and also distributed printed materials to them. In addition, the Office cooperated with voluntary organizations and the United States Department of State in preparing a packet of information materials on the Soviet treatment for reporters and other individuals.

Special Requests

The Information Office continues to serve in an advisory capacity to the senior staff of the Institute. The Office is consulted about and/or handles the public information and press relations aspects of policy decisions; the preparation of scientific manuscripts, brochures, and audiovisual materials; and the response to Freedom of Information and Congressional requests. Assistance is also provided in drafting politically sensitive documents, including collaborative research agreements between the NEI and eye research organizations in other countries. In collaboration with the program planning staff, the Office drafted the NEI Director's Opening Statement before the House and Senate Appropriations Committees and subsequently reviewed and helped edit the transcripts of these hearings. The Office prepared the NEI's contribution to three special reports to Congress and also the annual Presidential proclamations for Save Your Vision Week and White Cane Safety Day.

INTRAMURAL RESEARCH

Clinical Branch

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1979 - September 30, 1980

REPORT OF THE CLINICAL DIRECTOR
Elmer J. Ballintine, M.D.

During the past year, the program of clinical research has continued smoothly despite the restrictions that have been imposed by the consolidation of facilities in the Clinical Center Inpatient Division, 13 West. The development and expansion of facilities for psychophysical and electrophysiologic testing under the direction of a single scientist was completed last year and has proved its value by providing efficient use of the clinical facilities and in demonstrating new correlations that are almost certain to lead to improvement in patient care. For example, it has been shown that in patients with anterior uveitis or peripheral chorio-retinitis, changes in the waveform of the electroretinogram can be detected reliably by newly developed methods days or weeks before any anatomic or visual acuity changes can be detected, and that this occurs in those patients who eventually have macular involvement from the uveitis. In another context, investigation of acquired color vision defects shows that there are at least two kinds of blue-yellow defects. These findings may be useful early in the course of the disease, in classifying various kinds of macular degeneration.

The Section on Retinal Ocular Connective Tissue Diseases has continued to study the defects of collagen, basement membrane, and proteoglycan metabolism in ocular diseases. The underlying principle for many of these methods is the incubation of tissue isolates with labeled precursors of the macromolecules composing the extracellular matrix. The intact macromolecules are extracted by guanadine hydrochloride solution and fractionated by molecular sieve chromatography. The isolated glycoconjugates are then characterized by enzymatic degradation and isolation of radiolabeled fragments.

In the past, investigators have enzymatically digested whole tissue; interpretation of the results has been ambiguous because it could not be determined into which of the macromolecular species the labeled metabolites had been incorporated.

The results of metabolic studies are being correlated with structural features by the use of mono-specific fluorescent antibodies to extracellular matrix components. These methods are being used to continue the investigations of the metabolic defects in corneal dystrophies.

These techniques have also been applied to the study of proteoglycans of normal and dystrophic corneal cells and to basement membrane synthesis in tissue culture. The presence of a collagenase, that is preferentially active against basement membrane collagen previously found only in tissue cultures of keratoconus corneas, was demonstrated in fresh keratoconus tissue by immuno-fluorescent specific antibody staining.

The insights to the molecular bases for a variety of degenerative ocular diseases being obtained in this laboratory suggest that practical application

of these results to diagnostic or therapeutic manipulation of the defective enzymatic machinery is not remote.

These same techniques have been adapted to study the mucopolysaccharides of the trabecular meshwork isolated from monkey eyes. They are being extended to the study of human surgical specimens obtained during the performance of trabeculectomy for the control of simple glaucoma. We think that these methods offer the present best hope for explaining the mechanism of the steroid induced elevation of intraocular pressure in susceptible subjects and for discovering the biochemical basis for the alterations in trabecular function in simple glaucoma.

Only a few patients with gyrate atrophy of the retina and choroid, when treated with pyridoxine, have a 30%-50% reduction in serum ornithine. In other patients, reduction of serum ornithine is being maintained by low arginine, low protein diet supplemented with amino acids. In one such patient maintained on the diet for 26 months, there was improvement in dark adaptation which has been maintained.

Families of patients are being studied with assays of ornithine amino transferase and ornithine decarboxylase in tissues and tissue cultures in an effort to further define the metabolic defect in pyridoxine responders and non-responders, and to identify heterozygous carriers of the disease. These results are encouraging for they offer hope for successful treatment, and the methods serve as a model for the study of other more common retinal degenerations.

A study of Cogan's Syndrome was completed. Early indications that a specific pattern of HLA antigens would be associated with this disease were not confirmed and no relationship to HLA type could be found. Two types of Cogan's syndrome could be distinguished. In the typical cases, young adults have a sudden onset of interstitial keratitis, symptoms of Meniere's disease and deafness. In ten percent of the patients, life threatening aortic insufficiency develops. Atypical Cogan's syndrome has vestibuloauditory dysfunction and ocular inflammation other than interstitial keratitis in association with elements of rheumatologic syndromes. Twenty percent of the patients have systemic vasculitis.

A protocol for treatment of medically uncontrollable open angle glaucoma by randomization of the patients either to trabeculotomy with the argon laser or to conventional trabeculectomy has been approved by the National Eye Institute Institutional Review Board and recruitment of patients has begun. This trial, is designed to determine unambiguously the value of the laser procedure.

The work on development of techniques for trabeculotomy using the Q-switched laser has been completed in monkeys and a protocol for a randomized trial comparing the effectiveness of the Q-switched technique to that of other forms of trabecular surgery is being developed.

An incidental benefit from the systematic study of groups of patients in whom a single diagnosis has been unequivocally confirmed is that errors in

medical literature that have been carried uncritically from one text to another are corrected. For example, in a cooperative study with NINCDS, it was found that, in contradiction to several citations, among sixty patients with Gaucher's disease, not one had a "cherry-red spot" in the macula, nor did biopsy of eight pingueculae reveal any Gaucher's cells.

Twenty-five trabeculectomy specimens from patients with primary open angle glaucoma or chronic angle closure were compared to specimens from eleven age-matched autopsy eyes by immunofluorescence and immunoperoxidase technique.

In the past, deposits of antibody-like materials in the meshwork of glaucomatous eyes has been reported. In these eyes, there were no deposits of Ig A, Ig M, Ig G, and the C₃ component of complement. These results do not indicate an important contribution from immune mechanisms to the glaucomatous defect in the trabecular meshwork.

Review of data accumulated over the past six years in the study of the pigment dispersion syndrome has revealed several characteristics of the accompanying glaucoma some of which are different from those of chronic simple glaucoma (CSG). As in CSG, a few cases occur in familiar clusters, but in contrast to CSG, there is no indication that the results of topical steroid testing and PTC testing are more than randomly associated with pigmentary glaucoma. The distribution of HLA antigens in the pigmentary dispersion patients is not different from that of the normal population.

In cooperation with the Clinical and Cellular Biology Branch, NIAMDD, a project was completed in which 44 acromegalic patients were examined ophthalmologically, and satisfactory retinal fluorescein angiograms and fundus photographs were interpreted. No elements of diabetic retinopathy were found among 29 patients with serum growth hormone elevated to an average five times the normal range for an average of 10.5 years. The fluorescein angiograms did not show any diabetic retinopathy that was not seen on the fundus photographs. These results do not support the hypothesis that diabetic retinopathy is caused by excess growth hormone and that diabetic retinopathy might be controlled by manipulating growth hormone mechanisms.

The Section on Clinical Eye Pathology processed one hundred eyes for standard histopathologic examinations. Forty eyes were used for research by other investigators in the Clinical Branch. Forty surgically excised tissues from patients were processed.

Three hundred animal eyes were processed for various research projects related to uveitis and drug effects on inflammation.

One hundred and fifty specimens were processed for transmission electron microscopy. One hundred and eighty-three tissue samples were processed for scanning electron microscopy.

There were 2,306 outpatient visits and 1,167 inpatient visits referred from other Institutes to the NEI clinical facilities. Forty-nine inpatients were admitted and thirty-two surgical operations were performed.

The Clinical Branch continued to cooperate with other NIH Institutes in pursuit of timely research opportunities. Patients under treatment for metastatic breast cancer are being monitored for ocular metastases and ocular toxicity of anticancer drugs. These drugs have been remarkably free of deleterious ocular effects, but recently, five patients receiving high doses of tamoxifen had reduced visual acuity, subepithelial corneal opacities, macular edema, and refractile white deposits in the retina. Tamoxifen-treated patients are being studied to determine if lower doses will also eventually produce the lesions.

In cooperation with the Southwestern Field Studies Section of NIAMDD, the results of a six-year follow-up study of the increased incidence of retinopathy in diabetic Pima Indians with elevated blood pressure was published.

The Clinical Branch provides a pediatric ophthalmology representative to the Inter-institute Genetics Group.

Clinical Branch staff scientists continued to serve as consultants to the National Institute on Drug Abuse, Interagency Committee on New Therapies for Pain and Discomfort, the Food and Drug Administration's Subcommittee on Ophthalmic Drugs, and the International Vitamin A Consultative Group.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00150-07 CB									
PERIOD COVERED October 1, 1979, to September 30, 1980											
TITLE OF PROJECT (80 characters or less) Ocular Hypertension Study											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Elmer J. Ballintine</td> <td style="width: 33%;">M.D. Clinical Director</td> <td style="width: 33%;">CB NEI</td> </tr> <tr> <td>Other: Douglas E. Gaasterland</td> <td>M.D. Chief, Section on Glaucoma</td> <td>CB NEI</td> </tr> <tr> <td>Richard Weiblinger</td> <td>B.S. Biologist</td> <td>CB NEI</td> </tr> </table>			PI: Elmer J. Ballintine	M.D. Clinical Director	CB NEI	Other: Douglas E. Gaasterland	M.D. Chief, Section on Glaucoma	CB NEI	Richard Weiblinger	B.S. Biologist	CB NEI
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Richard Weiblinger	B.S. Biologist	CB NEI									
COOPERATING UNITS (if any) Office of Biometry and Epidemiology, NEI											
LAB/BRANCH Clinical Branch											
SECTION											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205											
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4									
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) <p> Patients with <u>ocular hypertension</u> are randomly assigned to treatment with topical <u>pilocarpine</u> in one or both eyes or to no treatment. The objectives of the study are: 1) to determine if treatment with pilocarpine to reduce intraocular pressure before visual field changes occur will reduce the number of ocular hypertensive subjects who eventually become glaucomatous, and 2) to determine if measurements of aqueous humor dynamics, the response to water loading of diurnal variation in intraocular pressure, serial stereophotographs of the optic disc, and measurements of visual fields help to predict which patients will eventually become glaucomatous. </p>											

Project Description:

Protocol Number: 77 EI 38

Objectives: Prolonged observation of a series of patients with ocular hypertension, some of whom are treated with miotics, will help to determine which signs have value in predicting those who will eventually require treatment and to determine if early treatment of ocular hypertension has any value in preventing visual field loss or in slowing the rate of development of abnormalities of aqueous humor dynamics.

Methods Employed: A detailed plan for classifying patients with ocular hypertension; observing them by repeated examinations including measurement of visual fields, aqueous humor dynamics, and photogrammetry of the optic disc over a period of five or more years; and randomly assigning patients to treatment with pilocarpine collyria in one or both eyes, or to no treatment, has been standardized.

Major Findings: There has been no indication that the course of ocular hypertension has been affected by treatment. Over one hundred patients have been examined to determine eligibility, and thirty-two are under continuing observation following randomization to treatment groups.

Significance to Biomedical Research and the Program of the Institute: Early, precise identification of patients who require treatment because they are in the early stages of the simple glaucoma remains an unsolved problem. The data being collected in this study will furnish a basis for establishing criteria for treatment more precisely than is now possible. There is at present no detailed knowledge of the progression of optic disc changes in ocular hypertension. The data being collected in this study, as well as the development of better instruments for the measurements in this study, will supply needed information in this field.

Proposed Course: We expect that the project will continue for at least five years, and that 100 subjects will be randomized to the treatment groups.

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00099-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Search for Diabetic Retinopathy in Acromegaly		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Elmer J. Ballintine M.D. Clinical Director CB NEI Other: Phillip Gorden M.D. Chief, Clinical and Cellular Biology Branch DB NIAMDD		
COOPERATING UNITS (if any) Clinical and Cellular Biology Branch, NIAMDD		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.00
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>acromegaly</u> enrolled in NIAMDD Protocol No. 69 A 154 were examined ophthalmologically with special attention to visual field measurement to reveal <u>chiasmatic defects</u> , <u>ophthalmoscopic examination</u> to detect elements of <u>diabetic retinopathy</u> , and <u>fluorescein angiography</u> . Results have been collated with results of growth hormone assay, fasting blood sugar, and glucose tolerance testing. The results do not support the hypotheses that high serum concentrations of <u>growth hormone</u> predispose to diabetic retinopathy.		

Project Description:

Protocol Number: 69 A 154 (NIAMDD)

Objectives: Several investigators have speculated that diabetic retinopathy may be related to an excess of growth hormone. In the past, attempts to find diabetic retinopathy in acromegalics has usually failed. One reason may have been that the retinal examination methods were not sensitive enough to detect early changes. Retinal fluorescein angiography has been shown to detect early retinopathy in some eyes where it had been missed by other methods of examination. The large group of acromegalic patients under observation in NIAMDD Protocol No. 69 A 154 and the retinal fluorescein angiographic facilities of the Clinical Branch made it possible to seek early retinal changes in patients with acromegaly.

Methods Employed: Standard clinical examinations.

Major Findings: Of 52 acromegalic patients, 44 had satisfactory fluorescein angiograms, 4 or more ophthalmoscopic examinations, and at least one set of fundus photographs. Typical early diabetic retinopathy was found in only one patient, and he had longstanding diabetes. No elements of diabetic retinopathy were found among 29 patients with serum growth hormone elevated to an average 5 times the normal range for an average of 10.5 years. These findings do not support the hypothesis that elevated growth hormone will cause the retinal vascular changes seen in diabetes. A comparison of fluorescein angiography with stereo fundus photographs showed that no angiopathy was seen in the angiograms that was not seen on the fundus photographs.

Significance to Biomedical Research and the Program of the Institute: The prevention of diabetic retinopathy is a major objective of the NEI. The growth hormone hypothesis, if sustained, would suggest several therapeutic possibilities that might lead to clinical trials of pharmacologic agents which block growth hormone release or interfere with its action on target organs. This study suggests that such attacks on diabetic retinopathy are not likely to be successful. The results also indicate that in epidemiologic studies of early diabetic retinopathy, fluorescein angiography is not likely to increase the sensitivity of detection over that of stereo fundus photographs.

Proposed Course: Patient recruitment and follow up has been completed. A manuscript presenting the results of the study is being revised.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00022-06 CB																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Urokinase Central Retinal Vein Occlusion Trial																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
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LAB/BRANCH Clinical Branch																										
SECTION																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
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SUMMARY OF WORK (200 words or less - underline keywords)																										
<p> Patients with recent complete <u>occlusion</u> of the <u>central retinal vein</u> are randomly assigned to treatment either with intravenous urokinase followed by heparin, heparin alone or intravenous fluids alone. The patients are then examined periodically for one year, and the effectiveness of treatment is judged by restoration of vision and the degree of protection achieved against the development of <u>hemorrhagic glaucoma</u>. </p>																										

Project Description:

Protocol Number: 75 EI 100

Objectives: To determine if treatment with thrombolytic agent (urokinase) plus anticoagulation with heparin, or treatment by anticoagulation with heparin alone, is effective in reducing the loss of visual acuity and the progression to hemorrhagic glaucoma that is a consequence of occlusion of the central retinal vein.

Methods Employed: Patients are examined according to a detailed plan to determine eligibility for the study. Eligible patients, if they agree to participate, are assigned by randomization to one of three treatment plans:

(1) Twenty-four hours of continuous intravenous treatment with urokinase in an effort to resolve the occlusion of the central retinal vein. This is followed by two weeks of anticoagulation treatment with heparin to prevent reformation of venous obstruction.

(2) Heparin anticoagulation alone.

(3) Hospitalization and administration of intravenous fluids similar in volume to those used in the other treatment groups.

After the treatment period, the patients are examined periodically for one year to determine the rate at which hemorrhagic glaucoma occurs and the degree of restoration of vision to the eye.

Major Findings: Twenty patients have been examined to determine their eligibility and seven patients have been randomized to treatment. No trends have been observed.

Significance to Biomedical Research and the Program of the Institute: Occlusion of the central retinal vein is a serious cause of visual disability and one of its major consequences is hemorrhagic glaucoma, which almost invariably results in a blind, painful eye. In the past, treatment with anticoagulation has been advocated, but no convincing evidence of effectiveness has been published. With the development of an effective thrombolytic agent (urokinase), the possibility of dissolving the presumed cause of the obstruction, a thrombus in the central retinal vein, and the demonstration that urokinase is effective in thrombolytic disease in other sites support the decision to undertake this trial.

Proposed Course: Examination of published data on the course of occlusion of central retinal vein indicates that 75 patients will need to be recruited to demonstrate that a 50% improvement in vision is produced by the treatment. Recruitment has been slow, mainly because the present protocol requires two weeks hospitalization for each patient. The protocol is now being revised to shorten the period of hospitalization. We will continue to recruit until 75 patients have been treated.

NEI Research Program: Retinal and Choroidal Diseases -Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00063-02 CB												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Blue-Cone Function in Color Vision Defects														
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COOPERATING UNITS (if any) None														
LAB/BRANCH Clinical Branch														
SECTION														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:												
0.8	0.8	0.0												
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SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study <u>blue-sensitive cone</u> function in selected cases of <u>color vision defects</u> , with special emphasis on acquired defects. Subjects are examined with <u>electrophysiological</u> and <u>psychophysical tests</u> .														

Project Description:

Protocol Number: 79 EI 92

Objectives: To characterize and document color vision abnormalities mediated by dysfunction of blue-sensitive cones or their retinal pathways.

Methods Employed: Color vision is examined on the basis of a battery of psychophysical tests (increment thresholds, field and test spectral sensitivity of pi-mechanisms, spectral luminosity, chromagraph and saturation discrimination tests) and electrophysiological studies of cone responses.

Major Findings: Two varieties of acquired "blue-yellow" defects have been characterized to date. These defects differ in the spectral location of neutral points and in the color naming of short wavelengths; they also differ in the waveform of electroretinographic cone responses elicited with intense violet flashes on yellow backgrounds. Similar varieties of "blue-yellow" defects have also been observed in rare cases of congenital alterations of color vision.

Significance to Biomedical Research and the Program of the Institute: The results may help our knowledge of the mechanisms of acquired color vision defects which preferentially affect blue-sensitive cone function in cases of retinal insult or disease.

Proposed Course: Studies of blue-sensitive cone function in color vision defects will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Psychophysical Functions)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00059-02 CB																																										
PERIOD COVERED October 1, 1979, to September 30, 1980																																												
TITLE OF PROJECT (80 characters or less) Electrophysiological and Psychophysical Evaluation of Retinal Disorders																																												
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SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to provide <u>diagnosis or evaluation of toxic, inflammatory, degenerative, or congenital retinal disorders</u> , and to conduct tests and experiments directed towards the clinical application and development of <u>electrophysiological and psychophysical procedures</u> for measuring <u>visual function in patients</u> of NEI's Eye Clinic and of other services in the NIH Clinical Center.																																												

Project Description:

Objectives: Diagnosis or evaluation of visual function in toxic, inflammatory, degenerative, and congenital visual disorders affecting the retina. Development of clinical procedures for the study of visual function.

Methods Employed: Commercially available and laboratory-developed instruments are used in measuring visual function in normal volunteers and clinical patients on the basis of electroretinography (single flash and averaged Ganzfeld, averaged Focal), visually evoked cortical potentials, electroculography, sensory rod and cone thresholds, color vision testing, Stiles-Crawford effects, retinal image stabilization, visual perimetry and other psychophysical functions.

Major Findings: Psychophysical and electrophysiological evaluations were performed on about 250 patients for diagnostic purposes in collaboration with clinical associates and staff members of the NEI.

Present efforts are directed towards the development of new color vision tests and of a non-invasive system of retinal image stabilization for clinical procedures which would permit studies of focal electroretinography with very small stimuli at different retinal eccentricities, microperimetry, and psychophysical functions.

Significance to Biomedical Research and the Program of the Institute: The work has provided evaluations and diagnosis of retinal disorders in inpatients, outpatients, and referred patients of NEI's Eye Clinic at the NIH Clinical Center. Development of new research techniques and the application of new and existing research techniques to clinical procedures are expected to help improve the diagnosis of visual disorders and the understanding of physiopathological mechanisms of retinal disease.

Proposed Course: Electrophysiological and psychophysical studies of retinal disorders will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Psychophysical Function)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00065-03 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Physiological and Anatomical Studies of the Visual System of Primates		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Francisco M. de Monasterio Other: Stanley J. Schein Richard T. Marrocco Edna P. McCrane	M.D., D.Sc. M.D., Ph.D. Ph.D. B.S.	Visiting Scientist Guest Worker Visiting Scientist Biologist
		CB NEI CB NEI CB NEI CB NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> This project aims to study the anatomical and physiological organization of neurons of the visual system of non-human primates that can serve as a model for the human visual system. The project gives emphasis to the <u>chromatic and spatial properties</u> and <u>central projections</u> of neurons of the <u>retina</u>, <u>lateral geniculate body</u>, <u>striate cortex</u> and <u>extrastriate cortex</u> of macaque monkeys. </p>		

Project Description:

Objectives: To study the neural organization underlying the processing of visual data in retina and cortex, with particular emphasis on color.

Methods Employed: Intracellular and extracellular recordings from single neurons, intracellular staining with fluorescent dyes, extracellular recordings of mass responses; correlation of the distribution of single cell varieties and morphological cell types as seen by electron and light microscopy; autoradiography of the distribution of radionuclide-labelled neurons.

Major Findings:

I. Extracellular recordings from visual cortical neurons (Area V4):

In collaboration with Dr. Richard Marrocco, (of the Department of Psychology, University of Oregon, working here during a sabbatical visit) we have studied the spatial and spectral properties of visual neurons located in the extrastriate visual cortex. Recent studies of monkey prestriate cortex have reported an abundance of both color-opponent and color-biased cells in an area termed V4. The mechanisms underlying color opponency and color biasing in this broadly defined area are not well understood.

Extracellular recordings were made from anesthetized (N₂O) and paralyzed rhesus and cynomolgus monkeys. Horizontal penetrations were made into the anterior bank of the lunate-sulcus, the posterior bank of the superior temporal sulcus, and points in between. Penetrations were confined to the region within 10 mm of the tip of the inferior occipital sulcus. Averaged responses to moving and flashing stimuli were obtained on neutral and chromatic backgrounds photopically matched; the narrow-band test stimuli were equal-quantum or photopically matched.

Of 152 cells, 21% were visually unresponsive. The remaining cells were classed as color-opponent (CO, 3%), color-biased (CB, 19%), and non-color-coded (NC, 57%). Two varieties of CO cells were observed. Whereas both showed excitation to the onset of some wavelengths and opposite excitation to the offset of others, one variety also showed typical spectrally-opponent inhibition. CO cells were of the "red-green" or "magenta-green" opponent type. CB cells were strongly excited by some lights and weakly or not at all by others, including white. Most were red-biased while a few were blue- or magenta-biased; so far, no green-biased cells have been encountered. They do not have concealed color-opponency since an (inhibitory) opponent response was not disclosed by neutral or chromatic backgrounds. NC cells had roughly equal responses to photopically matched lights on neutral backgrounds, and selective chromatic adaptation did not disclose a color-opponent response. The spectral sensitivity of CO and CB cells was not unusually narrow-band but comparable to that observed at more peripheral centers. All cells had spatially-delimited receptive fields (typically smaller than 4-5° on the side) within the central 10° of the visual field; many were orientation-biased or directionally-biased. CO and CB cells were found adjacent to NC and visually-unresponsive cells of the same penetration. Also, any given penetration produced a mixture of cell types.

The results do not lend support to the notion that the area termed V4 is primarily devoted to color processing. Rather, they suggest that V4 may be functionally heterogenous, subserving luminance, movement and orientation, as well as color information.

II. Color opponency in the outer retinal pathway of blue-sensitive cones of the rhesus monkey.

Field potentials mediated by blue-sensitive cones of the monkey retina were obtained as the difference signal between responses to red and blue flashes stimulating red and green cones to the same extent. Comparison of these potentials with blue-cone potentials obtained by intense chromatic adaptation indicate that, in normal photopic conditions, signals from blue cones receive ON and OFF-transient suppression from green or red cone signals, or both. The transients are generated following the onset and offset of the late recetor potential of longer wavelength cones. These color-dependent interactions, taking place at the level of the outer plexiform layer, show that blue cone signals are sent along a color-opponent pathway rather than a blue-cone specific one. The results provide a retinal basis for several odd properties of the blue-sensitive mechanism of human color vision.

III. Intracellular recordings and staining of single retinal cells in the isolated monkey retina.

To understand the anatomical organization responsible for the physiological functioning of the primate retina, we are applying intracellular recording and staining techniques to identify functional cell types. We have obtained preliminary results that indicate the feasibility of this approach in the monkey retina.

IV. Sublayering in the inner plexiform layer of the monkey retina.

By applying conventional and new staining techniques we have examined a specific sublayering pattern that exists in the inner plexiform layer. This pattern corresponds to the level of termination of bipolar cell axon terminals of the monkey. By using different fluorescent tracers, the patterns appear to be connection-specific and, in development studies, it appears to follow known synaptic contacts between retinal cells. The results suggest that coupling between cones, if present at all, is very weak, and they provide interesting indications on the directionality of synaptic contacts between cones, bipolar and horizontal cells.

V. Physiological anatomy of the visual pathway.

Preliminary experiments have been performed in the study of functional anatomical connections using a 2-deoxy-glucose activity labelling method. This technique is being used in conjunction with newer histological staining procedures that will permit the study of the cyto- and myelo-architectonics of the visual cortex.

Significance to Biomedical Research and the Program of the Institute:

Understanding the organization of the visual system of non-human primates is valuable for the understanding of the human visual system, which at present can only be studied by indirect methods. Radionuclide-label studies appear to be one of the most promising approaches in this direction, because autoradiographic studies can be substituted by non-invasive methods of mapping the distribution of a positron-emitter nuclide.

Proposed Course: Both extracellular and intracellular recordings from single cells of the monkey visual system, as well as neuroanatomical studies of the system, will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation; Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

de Monasterio FM, Schein SJ: Protan-like spectral sensitivity of foveal Y ganglion cells of the retina of macaque monkeys. J Physiol (Lond) 299:385-396, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00061-02 CB																		
PERIOD COVERED October 1, 1979 to September 30, 1980																				
TITLE OF PROJECT (80 characters or less) Retinal Function in Posterior Uveitis																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Francisco M. de Monasterio</td> <td style="width: 15%;">M.D., D.Sc.</td> <td style="width: 20%;">Visiting Scientist</td> <td style="width: 10%;">CB</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Robert Nussenblatt</td> <td>M.D.</td> <td>Senior Staff</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td></td> <td></td> <td>Ophthalmologist</td> <td></td> <td></td> </tr> </table>			PI:	Francisco M. de Monasterio	M.D., D.Sc.	Visiting Scientist	CB	NEI	Other:	Robert Nussenblatt	M.D.	Senior Staff	CB	NEI				Ophthalmologist		
PI:	Francisco M. de Monasterio	M.D., D.Sc.	Visiting Scientist	CB	NEI															
Other:	Robert Nussenblatt	M.D.	Senior Staff	CB	NEI															
			Ophthalmologist																	
COOPERATING UNITS (if any) None																				
LAB/BRANCH Clinical Branch																				
SECTION																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																				
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0																		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords) Abnormalities of retinal function at the level of <u>rods</u> and <u>cones</u> or their <u>pathways</u> are being documented by <u>electrophysiological</u> and <u>psychophysical</u> studies of <u>patients</u> with <u>posterior uveitis</u> of suspected <u>immunological origin</u> .																				

Project Description:

Protocol Number: 79 EI 49

Objectives: To understand the retinal physiopathology of posterior-segment uveitis and chorio-retinitis of suspected immunological origin.

Methods Employed: Retinal function is assessed by electroretinography (single flash and averaged Ganzfeld responses, focal responses), electrocu-
lography, sensory dark-adaptation thresholds, visual perimetry and color
vision tests in cases of ocular toxoplasmosis, par planitis, Behcet's disease,
ocular sarcoid, Vogt-Kayanagi-Harada's syndrome, ocular histoplasmosis and
other inflammatory diseases affecting the posterior segment of the eye.

Major Findings: Studies of more than 100 cases of posterior uveitis
indicate that diffuse and central involvement of the retina produces early
electroretinographic waveform changes of cone responses. These changes,
primarily involving responses mediated by signals from red- and green-
sensitive cones, are accompanied by reduction or extinction of responses
mediated by signals from blue-sensitive cones. These alterations, which
appear to be an accurate diagnostic criteria to detect inflammatory activity
of immune origin, are accompanied by relatively typical, though unspecific,
color vision defects of central vision.

The observed changes in electroretinography, coupled with color vision
alterations and other psychophysical findings represent a nearly pathognomonic
sign of central retinal involvement which seems to be associated with cell-
mediated responses to the retinal S-antigen. These results have been confirmed
using S-antigen to generate experimental uveitis in rhesus monkeys.

Significance to Biomedical Research and the Program of the Institute:
The detected electrophysiological signs serve to study the clinical evolution
of the cases with diffuse central uveitis with retinal involvement using
comparatively simple tests. Characterization and localization of disordered
retinal function may elucidate some of the physiopathological processes of
immunological retinal disease.

Proposed Course: Studies of retinal function in posterior uveitis will
be continued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory
Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00006-09 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Research in Methods of Evaluating Visual Processes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Ralph D. Gunkel Other: David G. Cogan Fred C. Chu Douglas Reingold	O.D. M.D. M.D. M.S.	Ophthalmic Physicist Medical Officer Senior Staff Fellow Biologist
		CB NEI CB NEI CB NEI CB NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> The main thrust of this project has been to monitor the visual efficiency of eye patients while conducting tests, research, and experiments directed toward improving, replacing, or standardizing the procedures involved. These <u>psychophysical tests</u> consist mainly of <u>threshold measurements of color and visibility</u> under different conditions. They are necessarily subjective measurements, but the degree of subjectivity has in some cases been effectively reduced to make them more useful in diagnosis, treatment, and prognosis of known or suspected ocular abnormalities. </p>		

Project Description:

Protocol Number: 80 EI 08

Objectives: To discover and utilize the most effective and least traumatic methods for quantitating and evaluating changes in the eye or its adenexae brought about by disease, toxic materials, or degenerative processes. The ultimate goal would be the attainment of objective methods for collecting information which will contribute toward the maintenance or restoration of normal visual function.

Methods Employed: Conventional ophthalmic instruments and those developed here are used in measuring rod thresholds, cone thresholds, color thresholds, and related ocular functions in clinic patients. These psychophysical tests were done on 497 patients during the past year, including normal controls, NEI patients, and referrals from other NIH Institutes.

Major Findings: A patient's estimate of his own night vision, daytime vision, or color discrimination is often much different from its most credible measured value. Careful measurement of cone thresholds appears to be our most sensitive index for visual efficiency and its variations due to disease or drug ingestion.

Perception of colors in dyes and pigments is quite different from the perception of spectral or projected colors, especially in subjects known to have defective or anomalous color vision. The Chromagraph appears to provide the most reliable information.

Some subjects with severe color defects are unable to adapt to trades or professions where good color discrimination is important, which emphasizes the need for good color testing in vocational guidance.

Changes in color perception are very common in elderly subjects and in subjects with certain ocular or systemic abnormalities. The Chromagraph, which we introduced in 1976, appears to reveal and measure these changes earlier than any of the other test methods.

The Chromagraph also plots congenital anomalies in a way which makes them easily understandable to the subject as well as to the examiner.

Significance to Biomedical Research and the Program of the Institute: Psychophysical measurements provide one means for monitoring the state of toxic and degenerative retinopathies, and they are frequently considered in the medical management of clinic patients.

The Newtonian concept of the chromaticity circle, as materialized in the Chromagraph, has removed some of the mystery and confusion surrounding the subject of color vision and its variations.

A paper correlating certain types of defects in color vision found by the Chromagraphic method with specific disease entities was prepared for the Annual Meeting of the American Academy of Ophthalmology.

A paper comparing the Chromagraphic findings in severe congenital anomalies with the results of the conventional color tests was prepared for the Meeting of the Association for Research in Vision and Ophthalmology.

A number of devices and accessories were constructed or suggested for other investigators.

Proposed Course: Since a protocol for "Measurement of Color Vision" has been approved, it will probably incorporate most, if not all, of the activity which would have been included in this project.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00085-03 CB																														
PERIOD COVERED October 1, 1979, to September 30, 1980																																
TITLE OF PROJECT (80 characters or less) The HLA and ABO Antigens and Immunologic Studies in Cogan's Syndrome																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">PI:</td> <td style="width: 35%;">Muriel I. Kaiser-Kupfer</td> <td style="width: 15%;">M.D.</td> <td style="width: 15%;">Senior Staff Ophthalmologist</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>David G. Cogan</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Kamal K. Mittal</td> <td>Ph.D.</td> <td>Research Microbiologist</td> <td>BB</td> <td>DBBP</td> </tr> <tr> <td></td> <td>Barton Haynes</td> <td>M.D.</td> <td>Staff Fellow</td> <td>LCI</td> <td>NIAID</td> </tr> <tr> <td></td> <td>Anthony Fauci</td> <td>M.D.</td> <td>Senior Physician</td> <td>LCI</td> <td>NIAID</td> </tr> </table>			PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	David G. Cogan	M.D.	Senior Staff Ophthalmologist	CB	NEI		Kamal K. Mittal	Ph.D.	Research Microbiologist	BB	DBBP		Barton Haynes	M.D.	Staff Fellow	LCI	NIAID		Anthony Fauci	M.D.	Senior Physician	LCI	NIAID
PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI																											
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COOPERATING UNITS (if any) Laboratory of Clinical Investigation, NIAID Bureau of Biologics, Food and Drug Administration																																
LAB/BRANCH Clinical Branch																																
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SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this protocol is to determine the phenotype frequency of the <u>HLA and ABO antigens</u> as well as to explore the possibility of <u>altered immune</u> <u>response</u> in patients with <u>Cogan's syndrome</u> .																																

Project Description:

Protocol Number: 77 EI I 138

Objectives: To determine the HLA and ABO antigens in patients with Cogan's syndrome. To determine in vitro immunologic studies on serum, blood, or separated mononuclear cells.

Methods Employed: Patients having Cogan's syndrome are examined according to a standard set of procedures to confirm the diagnosis. Blood specimens are analyzed for HLA and ABO antigens and a prescribed battery of in vitro immunologic studies.

Major Findings: Patients with Cogan's syndrome do not have a specific HLA type. As a result of this study, a classification of Cogan's syndrome has been possible. Typical Cogan's syndrome is a disease of young adults characterized by flare-ups of interstitial keratitis, sudden onset of Meniere's-like attacks and deafness. The prognosis of typical Cogan's syndrome is excellent with only 10% of the patients developing life-threatening aortic insufficiency. Atypical Cogan's syndrome (vestibuloauditory dysfunction with ocular inflammation other than interstitial keratitis) overlaps with other rheumatologic syndromes and carries a less favorable prognosis being associated with vasculitis in 21% of patients. Treatment of interstitial keratitis consists of topical corticosteroids, and a short trial of systemic steroids is warranted as soon as possible after the onset of hearing loss.

Significance to Biomedical Research and the Program of the Institute: To determine the immunologic basis of an eye disease, clarify prognosis and recommended treatment.

Proposed Course: Project to be terminated.

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Diseases

Publications:

Haynes B, Kaiser-Kupfer MI, Mason P: Cogan's Syndrome: Studies in thirteen patients, long term follow up, and a review of the literature. Medicine (in press).

Project Description:

Objectives: To determine in patients placed on tamoxifen the minimum level at which ocular changes are noted.

Methods Employed: All NCI metastatic breast carcinoma patients placed on tamoxifen are examined ophthalmoscopically. In addition, psychophysical testing including color vision testing, cone thresholds, and dark adaptation are performed. When appropriate, fundus photographs are taken. Patients are reevaluated periodically, depending upon the total dosage achieved.

Major Findings: Ocular toxicity of tamoxifen has been discovered in five patients on high-dose tamoxifen for prolonged periods.

Significance to Biomedical Research and the Program of the Institute: If the safe minimum level of tamoxifen can be recognized, then patients on this drug will not need to be monitored until such a dosage is reached.

Proposed Course: The project will continue for one additional year.

NEI Research Program: Retinal and Choroidal Diseases--Special Areas of Future Interest (Toxic and Environmental Disorders)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00083-03 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) The Pathogenesis of Gyrate Atrophy and Trial of Pyridoxine		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> PI: Muriel I. Kaiser-Kupfer Other: David Valle </div> <div style="width: 65%;"> M.D. Senior Staff Ophthalmologist CB NEI M.D. Assistant Professor, The Johns Hopkins School of Medicine </div> </div>		
COOPERATING UNITS (if any) Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS </div> <div style="width: 35%;"> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div style="width: 30%;"> <input type="checkbox"/> (c) NEITHER </div> </div>		
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>gyrate atrophy</u> of the <u>retina</u> are examined systematically to confirm the diagnosis. Skin fibroblasts grown in <u>tissue culture</u> are assayed for <u>ornithine aminotransferase</u> activity. Other enzymatic activities related to ornithine metabolism such as ornithine decarboxylase activity will be measured. The results will be examined for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given pyridoxine to see if the serum concentration of ornithine can be reduced, and if so, the patient will be classified as a "responder," and treatment with pyridoxine will be continued. Nonresponder and responder patients will be placed on a low arginine, low protein diet with supplemental amino acids and observed for an arrest or improvement of their disease.		

Project Description:

Protocol Number: 78 EI 01

Objectives: To determine the biochemical processes responsible for the elevated serum ornithine and the retinal lesion that occurs in gyrate atrophy of the retina. To determine which patients respond to pyridoxine treatment with a decrease in serum ornithine concentration. To determine if treatment of "responders" with pyridoxine and dietary manipulation will arrest the progress of the retinal atrophy.

Methods Employed: Patients suspected of having gyrate atrophy of the retina are examined according to a standard set of procedures to confirm the diagnosis. Serum ornithine concentration is measured periodically. Punch biopsies of the skin are grown in tissue culture, and their enzymatic activity related to ornithine metabolism is measured.

Major Findings: Patients with gyrate atrophy of the retina have been shown to have a deficiency of ornithine aminotransferase. A small percentage of patients with gyrate atrophy have a 30%-50% decrease of serum ornithine while on pyridoxine therapy. A single patient has been followed for 26 months on a low protein, low arginine diet and has been found to show an improvement in dark adaptation on this regime with lowered serum ornithine levels.

Significance to Biomedical Research and the Program of the Institute: Gyrate atrophy of the retina is the first of the genetically determined isolated severe retinal degenerations for which a specific biochemical concomitant defect has been demonstrated. The study will guide and test the efficacy of treatment for this blinding eye disease and serve as a model for the investigation of other genetically determined retinal degenerations.

Proposed Course: This project will be continued for three more years to further assess the knowledge of reduced ornithine in halting the chorioretinal degeneration.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Valle D, Walser M, Brusilow SW, Kaiser-Kupfer M: Gyrate atrophy of the choroid and retina: Amino acid metabolism and correction of hyperornithinemia with an arginine deficient diet. J Clin Invest 65:371-378, 1980.

Kaiser-Kupfer MI, Valle D, Bron AJ: Clinical and biochemical heterogeneity in gyrate atrophy. Am J Ophthalmol 89:219-222, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00011-06 CB						
PERIOD COVERED October 1, 1979, to September 30, 1980								
TITLE OF PROJECT (80 characters or less) Pigment Dispersion With and Without Glaucoma								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Muriel I. Kaiser-Kupfer</td> <td style="width: 33%;">M.D. Senior Staff Ophthalmologist</td> <td style="width: 33%;">CB NEI</td> </tr> <tr> <td>Other: Carl Kupfer</td> <td>M.D. Director</td> <td>NEI</td> </tr> </table>			PI: Muriel I. Kaiser-Kupfer	M.D. Senior Staff Ophthalmologist	CB NEI	Other: Carl Kupfer	M.D. Director	NEI
PI: Muriel I. Kaiser-Kupfer	M.D. Senior Staff Ophthalmologist	CB NEI						
Other: Carl Kupfer	M.D. Director	NEI						
COOPERATING UNITS (if any) None								
LAB/BRANCH Clinical Branch								
SECTION								
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205								
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2						
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to compare patients having <u>pigment dispersion syndrome with and without glaucoma</u> . The data acquired may enable a determination of the risk of patients with pigment dispersion syndrome to develop glaucoma as well as add to understanding of the pathology of the disease state.								

Project Description:

Protocol Number: 76 EI 189

Objectives: To compare patients having pigment dispersion with and without glaucoma by documenting and following the clinical features and course of their disease and by evaluating the patient's performance on a variety of diagnostic tests. To determine the presence of abnormal aqueous humor dynamics using provocative testing in patients having pigmentary dispersion with and without glaucoma. To compare pigment dispersion with and without glaucoma with respect to possible genetic markers. To determine whether pupillary responses to light stimulation are abnormal in cases having iris transillumination.

Methods Employed: At the first visit, the following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Applanation Goldmann tension (app)
Photography of iris transillumination
Goniophotography

At the next visit, the following examinations are performed:

Static perimetry
Base-line tonography and water-drinking tonography one hour later
Fasting blood sugar when indicated

At the third visit, the following examinations are performed:

Slit lamp photography of Krukenberg spindle
Dilated ophthalmoscopic examination (10% phenylephrine and 1% cyclogel)
Stereophotographs of the optic nervehead

At the fourth visit, pupillography is performed.

Major Findings: Patients may have pigment dispersion syndrome for as long as 20 years without developing glaucoma.

There may be a hereditary predisposition in some cases, as seen in a mother and daughter, two brothers, and a brother and sister.

Steroid testing and PTC taste testing do not appear to show any particular categorization of these patients. Recent evidence has indicated that HLA antigens in patients with pigment dispersion are also not significantly different than those in the normal population.

It may be noted that whether filtering procedures are performed or not, pigment may be lost from the trabecular meshwork in time.

Significance to Biomedical Research and the Program of the Institute:

These data may enable a determination to be made of the risk of patients having pigment dispersion to develop glaucoma. Specifically, it may be possible to identify which features of these determinations have predictive value in forecasting which of those patients having pigment dispersion will develop a visual field defect. In addition, the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma can be investigated.

Proposed Course: This project will be continued for three more years to continue to obtain data to further understand the knowledge about pigment dispersion syndrome.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00062-04 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Progressive Essential Iris Atrophy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI Other: Carl Kupfer M.D. Director NEI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .56	PROFESSIONAL: .44	OTHER: .12
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Patients are being recruited with progressive essential iris atrophy with or without associated corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process, to investigate <u>aqueous humor dynamics</u> in both affected and unaffected eyes, and to attempt to find <u>genetic markers</u> such as <u>HLA and ABO antigens</u> or physical correlates with the disease process.		

Project Description:

Protocol Number: 76 EI 219

Objectives: The objectives of the study are to develop a panel of patients with progressive essential iris atrophy and to study these patients to determine factors which may aid in understanding the pathophysiology of the disease process and to study the natural history of this disease. Measurements of aqueous humor dynamics, assessment of genetic markers such as HLA and ABO antigens and physical correlates, and iris fluorescein angiography to determine the role of the vasculature will be carried out.

Methods Employed: During the course of the evaluation, the following procedures are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Photography of iris and iris transillumination
Gonioscopy and goniophotography
Iris fluorescein angiography and photography
Baseline tonography
A complete medical and dental evaluation
Dilated ophthalmoscopic examination
Stereophotographs of the optic nervehead

Major Findings: Histopathologic and electron microscopic study of iris and trabecular meshwork tissue has not indicated any clues to the pathogenesis of the disease process.

An ultrathin corneal contact lens is useful in certain patients to prevent recurrent rupture of corneal bullae.

Significance to Biomedical Research and the Program of the Institute: These data may contribute to an understanding of pathophysiologic factors involved in the rare entity of progressive essential iris atrophy. In addition, a careful study of the progression of the disease from the earliest signs will clarify the significance of corneal involvement and the status of outflow channels which may add to the understanding of the mechanism of glaucoma.

Proposed Course: The project will continue for four more years in an effort to obtain more data regarding the pathophysiology of this process.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00060-04 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Visual Function and Ocular Pigmentation in Albinism		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI Other: None		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .20	PROFESSIONAL: .15	OTHER: .05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>hypomelanotic disorders</u> such as <u>ocular albinism</u> , <u>oculocutaneous albinism</u> , <u>Chediak-Higashi Disease</u> , <u>Hermansky-Pudlak Syndrome</u> and <u>iris trans-illumination defects</u> are being recruited to determine visual function and to evaluate its course over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.		

Project Description:

Protocol Number: 76 EI 207

Objectives: The objectives of the study are to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; to correlate the amount of nystagmus with visual acuity and iris pigmentation; to determine whether ocular pigmentation, visual acuity, and nystagmus change with age; and to identify the heterozygous state in family members.

Methods Employed: The following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity at near and distance with refraction
Slit lamp examination
Psychophysical testing including D-15 and Munsell 100
hue, rod and cone thresholds
Dilated ophthalmoscopic examination
Hair bulb incubation
Photography to document hair color, eye color, iris
transillumination, disc, and macula

Examination of family members includes:

Best corrected visual acuity
Slit lamp examination of iris
Photography of iris transillumination
Fundus examination when vision not corrected to 20/20

Major Findings: Examination of patients and family members indicates that the finding of transillumination of the iris may be seen in the absence of recognized albinism. The pattern appears to be punctate and may be present in a diffuse manner or limited to the 6 o'clock sector.

Significance to Biomedical Research and the Program of the Institute: These data may allow identification of the carrier state in albinism which would be of importance in genetic counselling. In addition, it may be possible to determine whether the development of the fovea is abnormal in albinism, and if this is the cause of the decreased visual acuity in albinism or whether decreased visual acuity is secondary to hypopigmentation and the resultant light-scatter and glare. In addition, it will be possible to ascertain whether visual acuity improves with age and if this is correlated with changes in pigmentation.

Proposed Course: This project will be continued for five more years in order to obtain additional data.

NEI Research Program: Retinal and Choroidal Diseases--Developmental
and Hereditary Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00084-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Carl Kupfer M.D. Director NEI Other: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.5	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) With recent embryological research indicating the role of the <u>neural crest</u> in contributing to all connective tissues anterior to the lens epithelium, the group of <u>developmental anomalies</u> of the anterior chamber with <u>glaucoma</u> or <u>ocular hypertension</u> are being reviewed.		

Project Description:

Protocol Number: 77 EI 119

Objectives: The objective of this study is to determine whether congenital and/or developmental anomalies of the anterior chamber are related to faulty migration or terminal differentiation of neural crest tissue.

Methods Employed: Patients of all ages with congenital and/or developmental anomalies of the anterior chamber are being examined clinically to determine involvement of cornea, trabecular meshwork, iris stroma, lens and ciliary body. When intractable glaucoma is present that cannot be controlled with medication, surgery will be performed and the specimens examined histologically.

Major Findings: It appears that in this group of anomalies of anterior chamber development there are pathological changes in one or several tissues derived from neural crest. These include corneal stroma, corneal endothelium, anterior iris stroma, Descemet's membrane, and trabecular meshwork endothelium.

Significance to Biomedical Research and the Program of the Institute: A better understanding of the pathogenesis of these glaucomas may help in improving diagnosis and treatment.

Proposed Course: Patients with other anomalies of the anterior chamber including congenital cataracts will be examined for abnormalities in tissue derived from neural crests.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma)

Publications:

Kupfer C, Kaiser-Kupfer MI: Observations on the development of the anterior chamber angle with reference to the pathogenesis of congenital glaucomas. Am J Ophthalmol 88:424-426, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00093-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Cataracts in Juvenile Guinea Pigs with Allergic Encephalomyelitis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Robert Nussenblatt M.D. Senior Staff Ophthalmologist CB NEI Other: Sanford Stone M.D. Head, Immunology Unit OSD NIAID		
COOPERATING UNITS (if any) Department of Pathology, Albert Einstein College of Medicine Office of the Scientific Director, NIAID		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.3	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Allergic encephalomyelitis is a central nervous system disease of immunologic origin. In juvenile strain 13 guinea pigs, cataracts developed during severe allergic (autoimmune) encephalomyelitis syndromes produced actively or by transfer of living lymph node cells from sensitized strain 13 donors. These lens changes were manifested bilaterally within a two-week period of active sensitization or transfer of sensitized cells. The morphologic in vivo appearance of these cataracts is similar to both the galactosemic induced and tryptophan deficiency cataract models. A better understanding of the etiology of these lesions not seen before in this entity in guinea pigs will help in understanding cataract formation in systemic disease.</u>		

Project Description:

Objectives: To investigate the etiology of cataract formation in young inbred animals which develop an acute autoimmune neurologic disease.

Methods Employed: Induction of allergic encephalomyelitis in juvenile strain 13 guinea pigs is accomplished in one of two ways. The first method for immunization of these animals is the injection of guinea pig spinal cord in complete Freund's adjuvant into multiple nuchal sites. A second method is the induction of the disease in strain 13 adults or juveniles with the subsequent transfer of immunologically active cells to the histocompatible juvenile animals.

Each animal is observed carefully for evidence of weight loss, urinary incontinence, hind-limb wasting, and cataracts.

Major Findings: The majority of juvenile strain 13 animals which were recipients of transfers of lymph node cells from histocompatible juvenile or adult donors showed bilateral cataracts. A large number of those actively immunized also manifested the same lesions. The opacities are first located in the cortex and have a doughnut appearance, with fully opacified lenses being the end result. These lesions did not appear in nonhistocompatible guinea pig recipients.

Significance to Biomedical Research and the Program of the Institute: Cataract formation in guinea pigs has never been reported before with induction of this well-known immunologic model. This cataract model could provide an understanding of how systemic diseases may alter the ocular environment so as to induce lenticular opacities.

Proposed Course: We will study the biochemical basis for the lens changes and attempt to prevent the induction of these cataracts during the disease.

NEI Research Program: Cataract--Cataract Induced by Drugs and Radiation and Occurring Secondary to Other Eye Disorders

Publications:

Raine CS, Tranpott U, Nussenblatt RB, and Stone SH: Association of optic uveitis with chronic relapsing experimental allergic encephalomyelitis: Relevance to multiple sclerosis. Lab Invest 42:327, 1980.

Stone SH, Nussenblatt RB, Cross FL, and Raine CS: Cataracts and allergic encephalomyelitis: Acute opacification of the lens in paralyzed juvenile guinea pigs. Ophthalmic Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00092-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) HLA, ABO, and B-cell Alloantigens and Ocular Inflammatory Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Robert Nussenblatt M.D. Senior Staff Ophthalmologist CB NEI Other: Kamal K. Mittal Ph.D. Research Microbiologist BB FDA		
COOPERATING UNITS (if any) Bureau of Biologics, FDA		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>ocular toxoplasmosis</u> , <u>pars planitis</u> , <u>sarcoidosis</u> , <u>Behcet's disease</u> , <u>chorioretinitis of unknown origin</u> , and <u>birdshot choroidopathy</u> are being studied to determine the phenotype frequency of the <u>HLA</u> , <u>ABO</u> , and <u>B-cell alloantigens</u> . Since the B-cell alloantigens or <u>DR antigens</u> are thought to play a role in the <u>immunologic response to antigens</u> , these findings will complement other immune uveitis studies being simultaneously carried out.		

Project Description:

Protocol Number: 79 EI 48

Objectives: To determine whether ocular inflammatory disease manifests specific HLA or B-cell alloantigens more frequently than the average population.

Methods Employed: Heparinized blood samples from patients are subjected to microcytotoxic tests to determine the HLA and B-cell antigens. The ABO system is evaluated utilizing an anti-sera method.

Major Findings: HLA-B8 has been found to be associated with iridocyclitis in Black Americans. This antigen has been associated with a wide range of autoimmune diseases, and its presence in patients with this disorder strongly suggests a similar mechanism for this disease.

Significance to Biomedical Research and the Program of the Institute: The role of HLA and B-cell alloantigens in the immune response is only beginning to unfold. This study will indicate whether these alloantigens play a role in the ocular immune response.

Proposed Course: This study will continue in order that sizeable populations of various ocular immune entities will be studied.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB: HLA and ocular diseases: A review of four entities with close HLA associations. Immunology of the Eye, Workshop I, Immunogenetics and Transplantation Immunity. Immunology Abstracts (in press).

Nussenblatt RB, Mittal KK: Association of anterior uveitis in American blacks with HLA-B8, and not B27, in Terasaki PI (ed): Histocompatibility Testing. University of Southern California Press, 1980 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00075-03 CB																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Immune Functions in Ocular Diseases of Obscure Etiology																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Robert Nussenblatt</td> <td style="width: 15%;">M.D.</td> <td style="width: 25%;">Senior Staff Ophthalmologist</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Igal Gery</td> <td>Ph.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Stanley Cevario</td> <td>B.S.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Mario Salinas-Carmona</td> <td>M.D.</td> <td>Visiting Fellow</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI		Stanley Cevario	B.S.	Biologist	CB	NEI		Mario Salinas-Carmona	M.D.	Visiting Fellow	CB	NEI
PI:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI																					
Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI																					
	Stanley Cevario	B.S.	Biologist	CB	NEI																					
	Mario Salinas-Carmona	M.D.	Visiting Fellow	CB	NEI																					
COOPERATING UNITS (if any) Department of Ophthalmology, University of Louisville, Louisville, Kentucky Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, Maryland																										
LAB/BRANCH Clinical Branch																										
SECTION																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.5	OTHER: 0.8																								
CHECK APPROPRIATE BOX(ES)																										
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																										
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords)																										
<p> <u>In vitro cellular immune functions</u> are being studied in a masked method in patients with <u>ocular toxoplasmosis</u>, <u>presumed ocular histoplasmosis</u>, <u>pars planitis</u>, <u>Behcet's disease</u>, <u>ocular sarcoid</u>, <u>birdshot chorioidopathy</u>, and <u>chorio-retinitis of unknown origin</u>. Crude ocular antigens as well as the purified uveitogenic <u>soluble antigen (S-antigen)</u> of the retina are being used in a <u>lymphocyte microculture</u> technique in order to evaluate the presence of cellular immune memory to ocular tissues. Immune memory is also evaluated by the production of <u>lymphokine</u> in a <u>capillary migration system</u>. A <u>subgroup of patients</u> with posterior uveitis has been identified as having this immunologic memory. Other studies concentrate on the presence of <u>suppressor cell activity</u> and functioning of <u>macrophages</u> in these patients. These results shed light on the basic mechanisms of uveitis and may be used as a guide for specific immunologic therapy. </p>																										

Project Description:

Protocol Number: 79 EI 49

Objectives: The objective of this study is to investigate several immunological factors in ocular inflammatory disease and how they may relate to the course and chronicity of this disease. The identification of groups with specific immunologic alterations provide us with a more rational approach to therapy.

Methods Employed: The ophthalmic examination of all patients includes slit lamp examination, visual field tests, electroretinogram, and fluorescein angiography. Lymphocyte cultures are prepared using the microculture technique, where the immune cells are tested against various crude ocular extracts, as well as purified human bovine S-antigen, in order to assess evidence of cellular immune memory which is considered to be the in vitro equivalent of the anamnestic response in vivo. The capillary migration system is used to evaluate migration inhibition of macrophages, a test considered as an in vitro equivalent of lymphokine production in vivo. Suppressor cells from patients during latent and active ocular disease are induced in the laboratory by using concanavalin A, with their suppression capabilities tested in vitro in the presence of fresh responder cells and mitogens. Suppressor cell activity is also evaluated by the use of suboptimal doses of concanavalin A in culture, as reported by Bresnihan and Jasin (J Clin Invest 59:109, 1977). Macrophage activity is studied by examining their production of lymphocyte activating factor. Monoclonal antibodies to T cell subsets are, in addition, being used in an attempt to identify alterations in lymphocyte sub-groups.

Major Findings: A subpopulation of patients with ocular inflammatory disease manifested a positive "memory" response to the S-antigen. Positive responders appear to be those with active or inactive retinal lesions, and patients with various diseases were found to respond. It therefore appears that similar immune groups are present in different clinical entities.

Some patients with posterior uveitis respond to crude retinal extracts but not to the S-antigen, indicating the possible role of other retinal antigens still to be purified.

Posterior uveitis patients manifested increased Con A induced suppression when compared to controls. But these same patients had decreased suppression when measured by the method described by Bresnihan and Jasin.

Significance to Biomedical Research and the Program of the Institute: Uveitis is the cause of five percent of legal blindness in the United States. This is the first time that patients' immune cells have been shown to manifest cellular immune memory to a purified retinal antigen.

The grouping of patients with uveitis on the basis of specific immunologic functions or alterations may provide a more rational basis upon which to develop specific immunotherapy. Elucidation and treatment of inflammatory conditions of the eye are major interests of the NEI.

Proposed Course: This continuing study will focus on the posterior uveitic entities in order to investigate further the role of the S-antigen in each of these, and what, if any role abnormal suppressor cell activity may play.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Gery I, Ballintine EJ, and Wacker WB: Cellular immune responsiveness of uveitis patients to retinal S-antigen. Am J Ophthalmol 89:173, 1980.

Nussenblatt RB: Uveitis: The role of immunity. Palestra Oftalmologia Panamericana (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00094-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Immune Mechanisms in Experimental Autoimmune Uveitis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Robert Nussenblatt Other: Igal Gery Mario Salinas Toichiro Kuwabara Merlyn Rodrigues Stanley Cevario Francisco de Monasterio	M.D. Ph.D. M.D. M.D. M.D. B.S. M.D., D.Sc.	Senior Staff Ophthalmologist Visiting Scientist Visiting Fellow Head, Section on Experimental Pathology Head, Section on Clinical Eye Pathology Biologist Visiting Scientist
		CB NEI LVR NEI CB NEI LVR NEI CB NEI CB NEI CB NEI
COOPERATING UNITS (if any) Department of Ophthalmology, University of Louisville, Kentucky		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Guinea pig strain 13 animals, Lewis rats, and non-human primates immunized</u> <u>at a site distant to the eye with the Soluble antigen (S-antigen) of the retina</u> <u>in complete Freund's adjuvant develop experimental allergic uveitis (EAU).</u> Depending on the antigen immunizing dose and the animal, the ocular lesions can vary from an iridocyclitis to a panuveitis. <u>Lymph node cells, nonadherent</u> <u>T-cells obtained from peritoneal exudate cells, and peripheral lymphocytes</u> <u>from immunized animals manifested significant cellular immune responses</u> whether measured by the <u>lymphocyte culturing technique</u> or by evidence of the production of <u>migration inhibition factor (MIF) of macrophages.</u> Ocular <u>electrophysiologic (ERG) alterations seen in non-human primates with</u> <u>S-antigen uveitis are similar to those seen in patients with posterior uveitis.</u> Cyclosporin-A, a newly introduced experimental immunosuppressive drug, has been found to be effective in preventing the onset of EAU.		

Project Description:

Objectives: We have previously reported that experimental uveitis may be induced in animals by immunization with a purified component of the retina (S-antigen). This study is designed to elucidate the basic immunologic mechanisms of this laboratory model for uveitis and how this model may be altered or regulated.

Methods Employed: Strain 13 guinea pigs, Lewis rats, and non-human primates are immunized with purified S-antigen in complete Freund's adjuvant in one hind footpad or the nuchal region. Evidence of ocular inflammatory disease is monitored via slit lamp and ophthalmoscopic examinations. After two to four weeks, lymph node, peritoneal exudates, or peripheral blood cells are collected and used for several cellular immune studies. Lymphocyte cultures are prepared in microtiter plates and are stimulated with S-antigen as well as other antigens. Other immune cells from immunized animals are mixed with isogenic macrophages in order to demonstrate the release of migration inhibition factor in the presence of S-antigen. Lewis rats immunized with the S-antigen are "protected" by daily injections of cyclosporin A. Antibodies are evaluated by gel diffusion, ELISA, and indirect hemagglutination techniques, and eyes are taken for histology.

Major Findings: Animals immunized with S-antigen develop obvious clinical anterior and posterior uveitis which is confirmed by histology. Animals with ocular disease manifest significant cellular immune memory responses when measured by lymphoproliferative and macrophage inhibition techniques.

The EAU model in non-human primates parallels closely the disease seen in some posterior uveitis patients. Initial evidence suggests that cyclosporin A therapy may alter the clinical response of immunized animals.

Significance to Biomedical Research and the Program of the Institute: Experimental autoimmune uveitis is the first uveitis model utilizing a purified retinal antigen. The mapping out of its immune mechanisms may lead to an improved understanding of human ocular inflammatory disease. Immunoregulatory models developed in this system may be utilized in future human clinical trials, including ultimately cyclosporin.

Proposed Course: To describe fully the underlying immune events in this disease, and to develop a successful protocol dealing with either specific or nonspecific suppression of the disease.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Gery I, Salinas-Carmona M, Kuwabara T, Wacker, WB:
S-antigen induced uveitis in primates and guinea pigs. Fed Proc 39:470,
1980.

Nussenblatt RB, Gery I, and Wacker WB: Experimental autoimmune uveitis; Cellular immune responsiveness. Invest Ophthalmol Vis Sci 19:686, 1980.

Nussenblatt RB, Gery I, Kuwabara T, de Monasterio FM, and Wacker WB: The role of the retinal S-antigen in primate uveitis. Immunology of the Eye, Workshop II, Autoimmune Phenomena. Immunology Abstracts (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00107-01 CB																				
PERIOD COVERED October 1, 1979, to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Suppression of Retinoblastoma Proliferation by Human Soluble Lymphocyte Factors																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">Mario Salinas-Carmona</td> <td style="width: 20%;">M.D. Visiting Fellow</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Robert Nussenblatt</td> <td>M.D. Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Paul Russell</td> <td>Ph.D. Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>John Hooks</td> <td>Ph.D. Research Microbiologist</td> <td>LOM</td> <td>NIDR</td> </tr> </table>			PI:	Mario Salinas-Carmona	M.D. Visiting Fellow	CB	NEI	Other:	Robert Nussenblatt	M.D. Senior Staff Ophthalmologist	CB	NEI		Paul Russell	Ph.D. Research Chemist	LVR	NEI		John Hooks	Ph.D. Research Microbiologist	LOM	NIDR
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	Paul Russell	Ph.D. Research Chemist	LVR	NEI																		
	John Hooks	Ph.D. Research Microbiologist	LOM	NIDR																		
COOPERATING UNITS (if any) Laboratory of Oral Medicine, NIDR																						
LAB/BRANCH Clinical Branch																						
SECTION																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																						
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.25	OTHER: .75																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Mononuclear cells</u> when stimulated with <u>concanavalin A</u> develop different biological activities, including <u>suppressor activity</u>. The objective of this work is to find out whether the inhibitory activity is mediated through soluble factors, and to characterize these factors' <u>biological</u> and <u>physiochemical</u> properties. We have found that the factors responsible for suppression are <u>non dialyzable</u>, <u>heat stable</u>, <u>resistant to pH2</u> treatment and inhibits proliferation of a variety of cells including <u>human lymphocytes</u>, <u>retinoblastoma cells</u> and <u>stromal keratocytes</u>. </p>																						

Project Description:

Objectives: Human mononuclear cells when stimulated with concanavalin A (Con A) develop different biological activities. Under specific culture conditions, and in the presence of that mitogen, some lymphocytes inhibit proliferation of fresh autologous or allogeneic lymphocytes. The mechanism by which those cells exert their suppressor activity is not known. The objectives of the present work is to investigate whether the inhibitory effect of Con A activated human lymphocytes is mediated through soluble factors, and if so to characterize their biological and physiochemical properties.

Methods Employed: Purified lymphocyte populations are stimulated with Con A for different periods of time. The resultant cell supernatants are sterilized by membrane filtration and tested against fresh allogeneic lymphocytes, retinoblastoma cells, and stromal keratocytes. Tritiated methyl thymidine uptake is used to assess cell proliferation. Biochemical methods such as membrane ultrafiltration, sieve chromatography, pH2 and enzyme treatment of crude supernatants as well as the semi-purified fractions are performed to determine some properties of the suppressor factors; bioassays such as interferon determinations are also done.

Major Findings: Supernatants from Con A stimulated peripheral human mononuclear cells produce 40-60% suppression of retinoblastoma cell proliferation in culture; T cell enriched fractions are active in producing the inhibitory effect as compared to the non-T cell fractions. The suppressor activity is non-dialyzable, heat stable (56 C x 45 '), and pH2 resistant. Type II interferon has also been found in the suppressor supernatants, but methods by which interferon activity can be abrogated has little or no effect on the inhibitory action of the supernatant.

Significance to Biomedical Research and the Program of the Institute: A purified suppressor factor from normal human mononuclear cells has been sought for some time. Its identification would be of great benefit in understanding basic mechanisms of immuno-regulation.

Proposed Course: Sieve chromatography and other biochemical techniques are being used in order to isolate the suppressor substance. The mechanism of the suppressor factors against target cells will also be investigated.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00108-01 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (60 characters or less) Induction of Ocular Inflammation by a Synthetic Mediator		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Amos Ben-Zvi Merlyn M. Rodrigues Igal Gery Elliott Schiffmann	M.D. M.D. Ph.D. Ph.D.
		Visiting Associate Chief, Section on Clinical Eye Pathology Visiting Scientist Research Chemist
		CB CB LRV LDBA
		NEI NEI NEI NIDR
COOPERATING UNITS (if any) Laboratory of Developmental Biology, National Institute of Dental Research		
LAB/BRANCH Clinical Branch		
SECTION Section on Clinical Eye Pathology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.3	OTHER: 0.2
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A <u>chemotactic synthetic peptide</u> N-formyl-methionyl-leucyl-phenylalanine (FMLP) an analog of bacterial products is injected into the rabbit cornea, skin and vitreous to induce a reaction resembling the " <u>Arthus phenomenon</u> ." Injection of FMLP induces edema and granulocytic infiltration in the cornea, conjunctiva and skin. Perivasculitis is also observed in the conjunctiva and skin. These histologic changes are compared with the inflammation induced by complement component C5a or by OVA in specifically immunized rabbits. The time course of the appearance, peak time, and subsiding inflammatory response is elevated for the cornea, vitreous and skin. All inflammatory reactions induced by FMLP, C5a and rechallenge with antigen (OVA) are inhibited by topical application and subconjunctival injection of dexamethasone, quíhacrine, analog of arachidonic acid (ETYA) and indomethacin, agents that inhibit different sites of chemotaxis of polymorphonuclear leukocytes (PMN's). We are determining whether the inflammation induced by FMLP would be inhibited by carbobenzoxy-phe-Met (Z-phe-Met) a <u>competitive inhibitor</u> of FMLP for the PMN's receptor in vitro.		

Project Description

Objectives: The wide spectrum of inflammatory reactions in the eye in various ocular diseases suggests that a variety of mediators may be responsible for the diversity of the morphologic changes. The synthetic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) is an analog of a naturally occurring bacterial product that induces leukocyte chemotaxis. This heat stable low molecular weight peptide interacts with a specific cell surface receptor and induces superoxide production, lysosomal enzyme release, phagocytosis, and aggregation of polymorphonuclear leukocytes. The availability of FMLP is permitting a direct approach to the study of inflammatory response that can be compared with an "Arthus-like phenomenon" without antigenic rechallenge. The aim of this study is to test the ability of FMLP to induce ocular inflammation; to compare its effect with "immune mediated" inflammation in two different ocular tissues, the cornea and vitreous; and to analyze the inflammatory process induced by different agents with regard to their modulation by various anti-inflammatory agents.

Methods Employed: New Zealand albino rabbits are used untreated or after being immunized with ovalbumin (OVA). As chemotactic agents in the untreated animals we use the synthetic peptide (FMLP) and the fifth component of the complement (C5a); in the treated animals we use rechallenge with antigen. The modulators of inflammation in this project are carboxybenzoxy-phe-Met, dexamethasone, eicosatetraenoic acid (ETYA) quinacrine and indomethacin. The injected tissues are the cornea, 3 mm medial to the limbus, the vitreous through the pars plana and the epidermis of the skin. Histopathologic evaluation of the inflamed and treated tissues is performed by light and electron microscopy.

Major Findings: The injection of FMLP into the cornea, vitreous and skin of albino rabbits shows that the peak of the inflammation occurs in a different time in each tissue, thus the reaction in the conjunctiva, cornea, skin and vitreous peaks at 2,3-6,4-6 and 24-48 hours respectively. The early inflammatory response consists mainly of PMN's, while the delayed vitreal response consists of 30-40% mononuclear cells as well as PMN's. The histologic findings and the time course of C5a induced inflammation are similar to that induced by FMLP and both show histologic similarity to the effect caused by rechallenge with OVA. All inflammatory reaction can be inhibited in varying degrees by dexamethasone, quinacrine, indomethacin and ETYA, but only the inflammatory reaction caused by FMLP can be inhibited by Z-phe-Met, a specific competitive inhibitor for the cell surface receptor.

Significance to Biomedical Research and the Program of the Institute: The initial demonstration of chemotaxis of leukocytes in vivo was in 1888 by a German ophthalmologist, Theodore Leber. The availability of pure synthetic chemoattractants and the methods for quantitating chemotaxis in vitro enables us to evaluate the inflammatory response in ocular tissue in vivo. By using this synthetic analog of a natural bacterial product we are able to induce an "Arthus-like syndrome" resembling the "Arthus phenomenon" induced by "immune mediators." This is a useful tool to study inflammation in general and

in the eye in particular.

Proposed Course: Evaluation of the effect of FMLP and its competitive inhibitor for the receptor Z-phe-Met. Further experiments will be done to establish the mode of action of FMLP in ocular tissue in comparison to C5a and OVA induced inflammation.

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Disease.

Publications:

Ben-Zvi A, Rodrigues M, Schiffmann E, Gery I: Induction of inflammation by synthetic peptide, in Immunology at the Eye Workshop III, Section on Inflammation and Allergy. Immunology abstracts (in press).

Ben-Zvi A, Rodrigues M, Schiffmann E, Gery I: Induction of inflammation by synthetic peptides. Fed Proc 31:319, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00096-02 CB												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Clinicopathologic Studies of Human Ocular Diseases														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Merlyn M. Rodrigues</td> <td style="width: 15%;">M.D.</td> <td style="width: 33%;">Chief, Section on Clinical Eye Pathology</td> <td style="width: 19%; text-align: right;">CB NEI</td> </tr> <tr> <td>Other: Patricia Donohoo</td> <td>M.S.</td> <td>Biologist</td> <td style="text-align: right;">CB NEI</td> </tr> <tr> <td>Joseph Hackett</td> <td>B.S.</td> <td>Biologist</td> <td style="text-align: right;">CB NEI</td> </tr> </table>			PI: Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB NEI	Other: Patricia Donohoo	M.S.	Biologist	CB NEI	Joseph Hackett	B.S.	Biologist	CB NEI
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COOPERATING UNITS (if any) Wills Eye Hospital, Philadelphia Department of Ophthalmology, Louisville														
LAB/BRANCH Clinical Branch														
SECTION Section on Clinical Eye Pathology														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5												
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SUMMARY OF WORK (200 words or less - underline keywords) Patients with localized ocular diseases or with ocular manifestations of systemic diseases are examined clinically, and photographic documentation is made of significant findings. Biopsy specimens or autopsy eyes from these patients are examined by scanning and transmission electron microscopy and histochemical stains. Studies are performed on patients with <u>glaucoma</u> , <u>ocular and adnexal tumors</u> , <u>vitreo-retinal membranes</u> , ocular manifestations of systemic diseases, and <u>laser-induced</u> ocular lesions. Histological studies are also performed on <u>normal human and rhesus monkey cornea</u> , <u>iris</u> and <u>trabecular mesh-work</u> and include scanning and transmission microscopy of tissue specimens as well as of tissue cultures.														

Project Description:

Objectives: Studies of the morphology of tissue specimens as well as cell cultures from normal and abnormal ocular tissues are essential for further insights into possible pathogenetic mechanisms of disease. The utilization of immunohistochemical methods and histochemical stains are also helpful in the diagnosis of certain conditions.

Methods Employed: Specimens are obtained from patients at the National Eye Institute as well as other ophthalmic centers in the United States. In most instances, specimens are processed by appropriate techniques for cell culture, histology, histochemistry, and electron microscopy. Selected specimens are frozen for special immunological studies. In other cases, routine histopathology is performed.

Major Findings:

I. Histologic studies of selected normal human ocular tissues

Scanning and transmission electron microscopy were performed on normal human cornea, iris and trabecular meshwork obtained from eye bank and autopsy eyes. Cell cultures of the iris and trabecular meshwork were also examined by the same methods.

Three book chapters were prepared for a text book of histology. These included scanning and transmission electron microscopy of the normal human cornea, iris, and developmental abnormalities of the cornea.

II. Diseases of the cornea

A. Corneal involvement in Darier's disease

Patients with Darier's disease (hyperkeratosis follicularis) have unusual peripheral, deep epithelial grouped opacities. Electron microscopy of corneal biopsies from two patients revealed irregular and moderate edema of the corneal epithelium with subepithelial granular material. The attachment complexes of the basal epithelium to Bowman's layer were absent.

B. Corneal manifestations in monoclonal gammopathy

One patient had bilateral superficial corneal stromal opacities that resembled Reis-Buckler dystrophy clinically. The other had deep stromal lesions. Electron microscopy revealed extracellular parallel linear deposits. Immunoelectrophoresis of serum and urine in both cases disclosed elevated Kappa light chains. X-ray and bone marrow examination were normal. In both patients, the corneal lesions were the first clue to the systemic disease.

III. Glaucoma

A. Primary open-angle glaucoma

Twenty-five trabeculectomy specimens from patients with primary open angle glaucoma or chronic angle closure glaucoma, and eleven age-matched controls were examined by immunofluorescence and immunoperoxidase techniques to determine the types of collagen, immunoglobulins, and the presence of factor VIII-related antigen in the human aqueous drainage channels. In the glaucoma cases in controls we demonstrated that the electron dense basement membrane-like material in the peripheral portion of the trabecular beams and in the juxta-canalicular meshwork, consists at least in part, of type IV collagen, a noncollagenous protein ("laminin") and fibronectin. Factor VIII-related antigen was demonstrated in conjunctival vessels of the control eyes. Schlemm's canal and the trabecular endothelial cells did not stain for factor VIII-related antigen in any of the specimens examined. No deposits of IgA, IgM, IgG, and the C3 component of complement were detected in the aqueous drainage channels.

B. Chandler's syndrome

Cases of Chandler's syndrome were characterized clinically by unilateral glaucoma, mild iris stromal atrophy, corneal endothelial dystrophy, and elevated intraocular pressure. They were examined by slit lamp microscopy and gonioscopy and had photographic documentation of the significant changes. Scanning and transmission electron microscopy of trabeculectomy and iridectomy specimens disclosed a downgrowth of degenerated corneal endothelium and Descemet's membrane across the inner uveal meshwork. The iris stromal changes were minimal and the corneal endothelial extension across the trabecular meshwork disclosed a moderate increase of microvilli, cytoplasmic blebs, and filopodial processes. Descemet's membrane was irregularly thinned and closely adherent to the inner uveal meshwork.

C. Glaucoma associated with endothelialization of the trabecular meshwork in two of the cases of posterior polymorphous dystrophy.

The cells lining the trabecular meshwork disclosed features of epithelial-like cells with desmosomal junctions, scant mitochondria and numerous microvillus projections. These cells were a direct extension from the corneal endothelium which also exhibited similar features.

D. Pseudoexfoliation glaucoma

This patient had unusual iris transillumination defects that differed from those described with this entity. The enucleated eye showed deposits of basement membrane-like material on the lens, iris, ciliary epithelium in the conjunctival and iris vessels, as well as in the anterior chamber. Scanning and transmission electron microscopy revealed 10 nm filaments of basement membrane-like material. These were most abundant in the iris and ciliary epithelium.

IV. Studies on ocular lesions associated with systemic diseases

Conjunctival biopsies from patients with Gaucher's disease showed elastoid degeneration without evidence of Gaucher cells.

Light and electron microscopy were performed on lesions from patients with midline granuloma, Bechet's disease, and allergic conjunctivitis.

V. Vitroretinal disorders

A. Vitreoretinal membranes

Vitreoretinal membranes were examined in culture from cases of retinal detachment, some associated with massive periretinal proliferation and others from patients with diabetic retinopathy. Scanning and transmission electron microscopy of the cell cultures disclosed cells of glial origin and others derived from retinal pigmented epithelium.

B. Corpora amylacea of the optic nerve and retina

These deposits were studied with histochemical stains and electron microscopy, and were shown to represent products of axonal degeneration.

Significance to Biomedical Research and the Program of the Institute: These studies are directly concerned with mechanisms involved in secondary glaucoma and corneal and conjunctival diseases, and ocular manifestations of systemic diseases.

Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Secondary Glaucomas); Corneal Diseases--External Ocular Infections and Inflammatory Diseases; Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Disorders.

Publications

Rodrigues M, Katz S, Foidart J, Spaeth G: Collagen Factor VIII antigen and immunoglobulins in the human aqueous drainage channels. Ophthalmology 87:337, 1980.

Rodrigues M, Phelps C, Krachmer J, Cibis G, Weingeist T: Glaucoma secondary to endothelialization of the anterior chamber angle: A comparison of posterior polymorphous dystrophy and Chandler's syndrome. Arch Ophthalmol 98:688, 1980.

Eiferman R, Rodrigues M: Unusual superficial stromal corneal deposits in IgG monoclonal gammopathy. Arch Ophthalmol 98:78, 1980.

Blackman H, Rodrigues M, Peck G: Corneal lesions in Darier's disease. Ophthalmology (in press).

Rodrigues M, Krachmer J, Miller S, Newsome D: Bilateral corneal crystal line deposits in benign monoclonal gammopathy. Arch Ophthalmol 97:124, 1979.

Rodrigues M, Streeten B, Spaeth G: The spectrum of Chandler's syndrome. A clinico-pathologic study. Proc XXIII Int Congr Ophthalmol p. 1491, 1978.

Rodrigues M, Waring G, Hackett J, Donohoo P: Histology of the normal human cornea, in Duane D and Jakobiec F (eds): Histology of the Eye Hagerstown, Harper and Row (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00078-03 CB																														
PERIOD COVERED October 1, 1979 to September 30, 1980																																
TITLE OF PROJECT (80 characters or less) Histopathology and In Vitro Characteristics of Human Corneal Dystrophies and Degenerations																																
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COOPERATING UNITS (if any) Department of Ophthalmology, University of Iowa																																
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SUMMARY OF WORK (200 words or less - underline keywords) Human <u>corneal dystrophies and degenerations</u> , which have been clinically documented are studied as keratoplasty specimens with histochemical stains, scanning and transmission electron microscopy, and immunologic techniques in an attempt to elucidate pathogenetic mechanisms. This approach has provided insight into cell-cell relationships in the normal and diseased states. Cell cultures performed in selected cases are examined by scanning and transmission electron microscopy. Tissue and cell culture studies have demonstrated in vivo proliferation of corneal cells including epithelialization of the endothelial layer in corneas of three patients with <u>posterior polymorphous dystrophy</u> . In patients with <u>macular corneal dystrophy</u> intracellular and extracellular accumulation of fibrillogranular material was observed in the corneal stroma, Descemet's membrane and corneal endothelium. The presence and production of collagen, glycoconjugates, and collagenase has been investigated with immunofluorescent, electrophoretic, and chromatographic methods. Electron microscopic studies were performed on <u>keratoconus</u> and <u>pellucid degeneration</u> .																																

Project Description

Objectives: The study attempts to combine detailed clinical and genetic studies of patients with human corneal diseases, particularly corneal dystrophies, in order to obtain further insight into the mechanisms of corneal opacification.

Methods Employed: Corneal specimens from transplant patients are divided into portions and used separately for light, scanning, and transmission electron microscopy. These data provide insight into the morphological appearance of the cells and the extracellular materials of the corneal layers. Other portions of the surgical specimens are placed into tissue and cell culture to allow examination of the morphology and biosynthetic activities of the cells of the three corneal layers. Indirect immunofluorescence has shown the range of collagen types present in normal and abnormal tissue. Column chromatography and electrophoresis provide information about the collagen and glycoconjugate biosynthetic patterns of abnormal tissues.

Major Findings:

A. Corneal Dystrophies: All four corneal buttons from patients with hereditary posterior polymorphous dystrophy had an admixture of epithelial-like and endothelial cells on the posterior surface of Descemet's membrane which is normally lined by a monolayer of endothelial cells. The epithelial-like cells were characterized by numerous microvillus projections, prominent desmosomal cell junctions, intracytoplasmic filaments, and scant mitochondria. The adjacent endothelial cells displayed gap junctional complexes, numerous mitochondria with horizontal disposition of cristae, and prominent Golgi. Cells cultured from the corneal endothelium exhibited a similar admixture of cells with epithelial-like and endothelial characteristics. The epithelial-like cells stained positive with antibody to human epidermal keratin, while the endothelial cells were unstained. Corneal tissue with lattice dystrophy stained positive for amyloid with Congo red and displayed dichroism. Immunofluorescence and biochemical studies are in progress to characterize the type of amyloid present. In patients with macular corneal dystrophy, corneal buttons were obtained from both eyes, examination revealed abnormal accumulation of glycosaminoglycan in the corneal stroma as well as in Descemet's membrane and corneal endothelium. The deposits were composed of fibrillogranular material and stained positive with stains for glycosaminoglycan.

B. Corneal Degenerations: Keratoconus specimens had the same range of collagen types as normal cornea, with predominantly type I collagen. Type III collagen was detected only in scarred areas. Radioactive labeling experiments on cultured cells from these corneas have demonstrated an elevated production of collagenase compared with the normal. Two patients with pellucid corneal degeneration showed thinned corneas inferiorly with no evidence of vascularization. Light and electron microscopy of a corneal button from each patient revealed irregularity of the epithelium in the peripheral thinned areas with a normal Bowman's layer in one case and focal dehiscences in the other. Marked thinning of the corneal stroma accompanied by the presence of a small number

of histiocytes was present peripherally in both cases. Descemet's membrane and endothelium were normal. Stromal collagen was normal in diameter and periodicity. In one case, CM-cellulose and ³S₂SDS gel profile of the collagens synthesized by these stromocytes in vitro (³H proline label) was similar to those of control corneas and keratoconus specimens. Collagenase activity levels in the culture medium from explanted pellucid tissue were comparable to or slightly higher than those observed in keratoconus. Pellucid degeneration may represent a peripheral form of keratoconus.

In vitro studies on normal human corneal and scleral collagens showed that human scleral collagen influences corneal collagen fibril formation. The collagenous component of connective tissues from many parts of the body including the eye is heterogeneous with respect to collagen types. Corneal collagen is largely type I with small amounts of types III and V. Scleral collagen, in contrast, has a very large proportion of type III with much larger diameter fibrils than those of cornea. We examined fibril formation in vitro of pepsin-acetic acid extracted and purified preparations of human ocular (types I and III) and placental (type V, a gift from Dr. J-M Foidart) collagens alone and in various combinations. Solutions of collagens were prepared in 0.15 M phosphate 0.01 M tris, gelled at 37°C, centrifuged into pellets, fixed in glutaraldehyde and examined by scanning and transmission electron microscopy. Fibril diameters varied with type III the largest (175 to 190 nm) and type I and V smaller (70 to 90 nm). Mixtures (1:1 v/v) of types I and III and I and V contained an intermediate sized fibril of about 140 nm plus some small and some large fibrils. Mixtures produced a marked heterogeneity of fibril diameters as compared with pure gels. These results reflect the collagen fibril diameter patterns seen in vivo in cornea and sclera, and demonstrate the important effect of collagen type mixture on uniformity and size of collagen fibrils.

Significance to Biomedical Research and the Program of the Institute:

The mechanisms of opacification and destruction of the cornea in a variety of human diseases must be understood for the improved diagnosis and classification of these entities. This may also lead to a more rational basis for the appropriate treatment of these visually disabling processes. A thorough knowledge of the genetic component of these disorders, if any, will aid in more effective and complete genetic counseling.

Proposed Course: Patient material will be entered into this combined study as it becomes available. Emphasis will be placed on elucidating pathogenic mechanisms in hereditary posterior polymorphous dystrophy, keratoconus, and lattice and granular dystrophies. The use of immunological techniques will be expanded to a wider variety of specimens.

NEI Research Program: Corneal Diseases--Corneal Edema, Transplantation and Corneal Stromal Injury and Repair.

Publications:

Rodrigues M, Sun T-T, Krachmer J, Vidrich A, Newsome D: Epithelialization

of the corneal endothelium in posterior polymorphous dystrophy. Invest Ophthalmol Vis Sci 19:832-835, 1980.

Krachmer J, Rodrigues M: Posterior keratoconus. Arch Ophthalmol 96:1867, 1978.

Waring G, Rodrigues M, Laibson P: Corneal dystrophies, I. Dystrophies of the Bowman's layer, epithelium and stroma. Surv Ophthalmol 23:71, 1978.

Waring G, Rodrigues M, Laibson P: Corneal dystrophies, II. Endothelial dystrophies. Surv Ophthalmol 23:147, 1978.

Rodrigues M, Waring G: Anterior and posterior corneal dystrophies, in Klintworth G, Garner A (eds.): Pathobiology of Ocular Diseases. New York, Mercel Dekker Co (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00050-04 CB																				
PERIOD COVERED October 1, 1979, to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Aqueous Humor Flow Measurement by Fluorophotometry																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Douglas E. Gaasterland</td> <td style="width: 20%;">M.D. Chief, Section on Glaucoma</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Lessie McCain</td> <td>R.N. Clinical Technician</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>John Baldinger</td> <td>B.S. Junior Assistant Health Services Officer</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Claude Cummins</td> <td>B.S. Biologist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Douglas E. Gaasterland	M.D. Chief, Section on Glaucoma	CB	NEI	Other:	Lessie McCain	R.N. Clinical Technician	CB	NEI		John Baldinger	B.S. Junior Assistant Health Services Officer	CB	NEI		Claude Cummins	B.S. Biologist	CB	NEI
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COOPERATING UNITS (if any) None																						
LAB/BRANCH Clinical Branch																						
SECTION Glaucoma Section																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																						
TOTAL MANYEARS: 0.55	PROFESSIONAL: 0.15	OTHER: 0.40																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) <p>The <u>aqueous humor flow in humans</u> is measured by determining the rate of loss of <u>fluorescein from the eye after iontophoresis</u> into the cornea in <u>normal volunteers</u> and in <u>patients with ocular hypertension or glaucoma</u>.</p>																						

Project Description:

Protocol Number: 77 EI 104

Objectives: This project is designed to measure directly aqueous humor flow in humans. This will be compared to calculated aqueous humor flow. The symmetry and reproducibility of measurements of aqueous humor flow in the two eyes of normal volunteers and of patients with either ocular hypertension or glaucoma are to be studied; medication effects will be assessed.

Methods Employed: A cylindrical piece of polyacrilamide gel is saturated with fluorescein solution. The gel is touched to the cornea, and fluorescein is deposited due to a small current provided by a dry cell battery. A photomultiplier tube with appropriate filters, mounted on a slitlamp biomicroscope, measures the total amount of fluorescein in the eye as well as the aqueous concentration. Illumination is provided by a chopped light source. The photomultiplier tube signal is fed to a tuned amplifier. The rate of loss of fluorescein from the eyes as a function of time yields the flow rate of aqueous humor.

Major Findings: FY 1980 was the third year of this project. The protocol was reviewed and updated.

The group at Mayo Clinic has presented a nomographic and algebraic expansion of the Jones and Maurice Method for handling fluorometric results during this year. The work in our laboratory has been modified to incorporate this simplification in our experimental method. We have prepared, and are testing, a computerized method of data reduction to flow values. This allows result calculation either by the Jones and Maurice Method II or by the Mayo (Coakes and Brubaker) simplified method. A comparison of accuracy using older data is in progress.

In five young normal volunteers, each of whom had at least four replicate determinations, the mean aqueous flow is about 3.1 ul/min with a range from 2.7 to 4.2 ul/min. In all but one, the symmetry of flow is striking; the last person had higher flow in her right eye on four of five occasions (mean is 4.2 compared to 3.2 ul/min).

In nine selected ocular hypertensive patients, each of whom had two replicate determinations, the mean aqueous flow in the right eyes is 3.2 ul/min and in the left eyes is 3.5 ul/min. For these same patients the flow calculated from the Goldmann equation, using measurements performed the same day as one of the fluorometric studies, was 1.4 ul/min. Thus ocular hypertension patients appear to have the same flow rate as young normal volunteers. The amount that fluorometric flow exceeds calculated outflow is probably accounted for by diffusion of fluorescein and by unconventional outflow.

Significance to Biomedical Research and the Program of the Institute: The aqueous humor flow rate is a primary determinant of the intraocular pressure. This accurate, safe, reproducible, noninvasive, direct determination

of the flow in humans under normal and pathological conditions is leading to increased understanding of glaucoma and hypotony.

Proposed Course: The studies will continue.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 000154-07 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Experimental Glaucoma in the Rhesus Monkey		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Other: None		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION Glaucoma Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this investigation is to study the <u>morphology</u> , <u>physiologic function</u> , and <u>pharmacologic responses</u> in the eye of the rhesus monkey in its <u>normal state</u> compared to its state when <u>experimental glaucoma</u> has been induced by argon laser photocoagulation of the <u>trabecular meshwork</u> .		

Project Description:

Objectives: To study physiologic function, pharmacologic responses, and morphology of the monkey eye after induction of glaucoma by argon laser photocoagulation of the trabecular meshwork. To compare observations to normal control eyes.

Methods Employed: Circumferential argon laser photocoagulation of the rhesus monkey trabecular meshwork causes sustained elevation of intraocular pressure to the range of 30 to 55 mmHg, the pressure range found in many humans with open-angle glaucoma. This is in contrast to the acute, short duration, very high pressure elevation (more than 65 mmHg, up to 95 mmHg) seen in most animal models for glaucoma. Outflow facility is evaluated by perfusion. Aqueous flow is determined by turnover of radioiodinated serum albumin injected into the anterior chamber. Retinal and optic nerve function are studied clinically and by autoradiography and morphologically to evaluate evidence of altered axoplasmic flow. The retina is also studied in cross section or by preparing whole-mounts of the tissue. Additional studies of the effect of less than circumferential argon laser photocoagulation have been started.

Major Findings: In FY 1980 the major activity in this project has been continued interest in the effect of less than circumferential coagulation of the trabecular meshwork.

Despite repeated partial circumferential coagulation, the eye of the monkey does not develop constant elevation of the intraocular pressure. Coagulation of one-half the circumference, repeated three times, is associated with pressures varying between normal and the high 20's. Coagulation of two-thirds to three-fourths the circumference, repeated three times, is associated with pressures varying between high normal and 40 mmHg. Clinically, the nerve fiber layer in the retina appears normal. Subtle variable, conical cupping of the optic disk develops, and is more pronounced during times of elevated pressure.

Significance to Biomedical Research and the Program of the Institute: This experimental glaucoma is the best model available for human chronic open-angle ("simple") glaucoma. Using this model allows close examination of the retina and optic nerve changes, with the promise of additional insight into the mechanism of loss of visual function in the patient with glaucoma.

Proposed Course: The project will continue with emphasis upon effects of treating less than the entire circumference of the trabecular meshwork.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00046-04 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Laboratory Studies of Aqueous Humor Dynamics		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Douglas E. Gaasterland Other: John A. Barranger Toichiro Kuwabara Yoshitaka Ohnishi Yujiro Ishikawa	M.D. Chief, Section on Glaucoma M.D. Chief, Clinical Section M.D. Chief, Section on Experimental Pathology M.D. Visiting Scientist M.D. Visiting Scientist	CB NEI DMNB NINCDS LVR NEI LVR NEI LVR NEI
COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS, NIH		
LAB/BRANCH Clinical Branch		
SECTION Glaucoma Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0.0
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SUMMARY OF WORK (200 words or less - underline keywords) Various projects are being carried out to clarify <u>intraocular fluid movement</u> <u>in rhesus monkeys</u> . A method has been perfected for spectrophotometric deter- minations of <u>ascorbic acid concentration</u> in ocular and systemic fluids. <u>Clinical, physiologic, and morphologic studies</u> of the ocular alterations following an insult from <u>intracarotid hyperosmotic mannitol</u> show transient alteration of clinical and physiological status, despite permanent disruption of the pigmented epithelial layer of the <u>pars plana</u> and <u>pars plicata</u> of the ciliary body.		

Project Description:

Objectives: This project is designed to examine the physiology of intra-ocular fluid movement under varied experimental conditions.

Methods Employed: Standard methods of cannulation and perfusion with non-invasive and invasive pressure measurements and with subsequent determination of volumes and flow by weight changes, dilution or turnover techniques have been used.

Major Findings: During FY 1980, a reliable, simple, spectrophotographic method for giving reproducible determinations of aqueous humor ascorbic acid concentrations has been perfected. This method determines the decoloration of a solution of excess 2,6-dichlorophenol-indophenol that occurs due to reduction of the dye when ascorbic acid is added. The studies have clarified how this technique can be applied to fresh 10 to 25 microliter samples of posterior or anterior aqueous. The concentrations in aqueous from normal eyes have been tested and found to be in agreement with published observations of concentration in monkey aqueous measured by titration techniques or high pressure liquid chromatography.

Repeated clinical examinations were done in rhesus monkeys from three days to as much as seven months after one, two, three, or four manipulations during which the monkey received intracarotid mannitol. The purpose was to search for ocular toxicity as part of an investigation of therapeutic manipulation of the blood-brain barrier. As previously observed with urea, the blood-aqueous barrier was disrupted by the treatment. Protein leakage starts immediately, and clinical evidence of moderate cellular and protein blood-aqueous barrier leakage can be detected in some animals for as long as five weeks. After the first manipulation, intraocular pressure decreases to 30% of pretreatment and the depression lasts about 12 weeks. After the fourth manipulation, the depression is to 60% of pretreatment and lasts about four weeks. Recovery of intraocular pressure parallels complete clearing of aqueous flare upon clinical examination. Three eyes three to six months after a single manipulation and two eyes one and one and one-half months after the fourth manipulation had an ascorbic acid concentration 10 to 20 times higher than normal monkey serum concentrations. This is a normal aqueous ascorbic acid concentration. Histopathologic examination demonstrated early disruption of the pigmented epithelium of the pars plana and pars plicata, with vacuole formation. This healed with partial replacement of the pigmented epithelium with pleomorphic, squamous looking cells. Thus the secretion of ascorbic acid into aqueous humor occurs normally despite an impressive loss of anterior segment pigmented epithelium. The manipulation does not appear to cause much alteration of barriers to ascorbic acid diffusion. No clinical or histopathologic evidence was found that the manipulation affects the lens, vitreous, retinal pigmented epithelium or retina during the observation period. Transient papilledema accompanied extreme hypotony, but had cleared by the time of histopathologic examination of the affected eyes, and there was no histopathologic evidence for optic nerve alteration.

Significance to Biomedical Research and the Program of the Institute:

The studies are elucidating normal dynamics of aqueous humor, as well as abnormal dynamics in experimentally induced situations, mimicking clinical problems. These studies are yielding information applicable to understanding and treating glaucoma and hypotony.

Proposed Course: These studies will continue, emphasizing aqueous humor inflow, outflow, and composition.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00168-05 CB																																																
PERIOD COVERED October 1, 1979, to September 30, 1980																																																		
TITLE OF PROJECT (80 characters or less) Laser Surgery for Glaucoma																																																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																																		
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COOPERATING UNITS (if any) Biomedical Engineering and Instrumentation Branch, DRS; Armed Forces Radiobiology Research Institute																																																		
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SUMMARY OF WORK (200 words or less - underline keywords) <p> The high energy and power of <u>lasers</u> offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, <u>iridotomy</u> and <u>trabeculotomy</u> are possible. This has importance for <u>glaucoma patients</u> because of the potential improvement of surgical outcome and reduced surgical morbidity. The aim of this project is a systematic evaluation of laser effects in <u>simian</u> (rhesus) <u>eyes</u> and the application of promising systems and procedures to human glaucoma eyes under controlled conditions. </p>																																																		

Project Description:

Protocol Number: 80 EI 91

Objectives: To develop workable laser systems for anterior segment surgery and to apply these systems to the normal monkey eye. To study the physiological and morphologic effects of laser energy upon monkey eyes. To apply favorable laser systems under controlled conditions to the treatment of glaucoma in humans.

Methods Employed: Instruments are being developed to meet the unique requirements of ophthalmic application. Standard laboratory physiologic and histopathologic (including SEM and TEM) techniques are employed to study laser effects. The NEI laser laboratory is equipped with a modified model 800 Coherent Radiation argon laser, which has been used for this and other projects, and a Q-switched ruby laser.

Major Findings: Instrument development has continued to be an important activity during FY 1980, the fifth year of this project. Monitoring of the duration of the Q-switched ruby laser pulses has been achieved by incorporation of a sensitive photodiode/oscilloscopic system to detect the duration of a small fraction of the pulse of light. An optical bench is employed to house the optical elements for monitoring the laser, and is being modified to accept the articulated arm delivered late in the year. The problem of non-linear effects of pulsed irradiation upon optic elements has been alleviated by beam expansion to 15 mm and by reducing the energy per pulse.

The study of the acute effects of Q-switched ruby laser on outflow resistance and anterior chamber angle morphology was completed. The energy spectrum studied ranged from 20 to 110 mj per pulse. Each monkey eye received three application spots. Treatment did not alter outflow facility from values determined in preliminary studies completed at least four months before the laser exposure. SEM documented well-defined punctures in the trabecular meshwork at the low energies and explosive disruption at the high energies. All levels of treatment caused alteration of nearby corneal endothelium; the higher energies caused local angle recession or iridocyclodialysis. A study of the chronic effects of Q-switched ruby laser has been started, using energies of 10 mj or less per pulse.

Using high speed photography (Hycam unit modified to fire the laser when running at about 12,000 frames per second) and pulse energies of 80 to 120 mj, it has been possible to demonstrate the interaction of laser pulses with the cornea and iris. The first event is a plume of tear film and epithelium which develops over 0.17 milliseconds, persists for about 0.26 milliseconds, and decays over another 0.17 milliseconds. At 83 microseconds after the start of plume formation a pressure interaction wave forms in the cornea; this persists, spreading like ripples on a pond, for 3 milliseconds. The light indents the cornea. Similar distortion, without plume formation, occurs in the iris.

The effects of argon laser glaucoma treatment on monkey eyes have been under study. Five eyes of five monkeys received circumferential treatment, as advocated in the literature for glaucoma patients. Two weeks to six months later, the treated and control eyes were perfused to determine total facility, then fixed for light and electron microscopy. In some eyes the total facility is reduced from normal; in some it is not different from normal; in none is it increased above normal. One eye had a transient high elevation of intraocular pressure, attributed to trabecular edema. SEM shows scarred pits regularly distributed in the trabecular meshwork; cross sections document obliteration of the normal angle structures at the site of treatment and preservation of normal-looking tissue in between the treatment sites.

A clinical trial protocol has been completed, reviewed, and approved for random assignment of patients requiring intervention for progressive glaucoma despite maximally tolerated medical therapy to either argon laser glaucoma treatment or trabeculectomy. The first patient has entered the study, and was assigned to trabeculectomy. He is now well-controlled without medications four months after the surgical procedure.

Two iridectomies were accomplished with ease using the argon laser in a patient in whom it was not possible to perform an iridotomy after three attempts with the Q-switched ruby laser.

A patient with a thin pupillary membrane that became opaque six weeks after planned extracapsular cataract extraction consented to an attempt at decission using the Q-switched ruby laser. One pulse of 70 millijoules focused upon the center of the membrane, and directed toward the equator of the eye, created an excellent optical opening which became larger as the edges of the opening retracted over the next week. He developed phakoanaphylaxis from the liberated lens material, requiring two weeks of intensive management for secondary elevation of intraocular pressure; this cleared completely. No more intervention is required.

Significance to Biomedical Research and the Program of the Institute: Conceivably, a physically-noninvasive laser system for anterior segment surgery might replace conventional invasive operative procedures for some types of glaucoma. This possibility is still being investigated.

Proposed Course: The project will continue. Instrument development will include adaptation of pulsed lasers to the operating microscope and the slitlamp using the articulated arm if possible. A frequency doubled ND/YAG Q-switched laser has been ordered. This will update the laser equipment and allow expansion of the studies to the green wavelengths. The clinical trial will be expanded to include Q-switched ruby laser and/or ND/YAG laser as adequate equipment becomes available.

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00143-07 CB												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Douglas E. Gaasterland M.D. Chief, Section on Glaucoma</td> <td style="width: 10%;">CB</td> <td style="width: 30%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Elmer J. Ballintine M.D. Clinical Director</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Carl Kupfer M.D. Director</td> <td></td> <td>NEI</td> </tr> </table>			PI:	Douglas E. Gaasterland M.D. Chief, Section on Glaucoma	CB	NEI	Other:	Elmer J. Ballintine M.D. Clinical Director	CB	NEI		Carl Kupfer M.D. Director		NEI
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COOPERATING UNITS (if any) None														
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TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <p> Thirty-six patients with <u>pigmented ocular lesions</u> originally participated in this study. The early results of the study show that the <u>diagnostic technique</u> used had <u>inadequate specificity</u>. For most patients a clear diagnosis has been made, and their ocular problem resolved. Except for occasional <u>follow-up examinations</u> of some of the patients, work on this project has ended. </p>														

Project Description:

Protocol Number: 76 EI 370

Objectives: To determine the value of using I-125 labeled chloroquine analog for the detection of ocular melanoma.

Methods Employed: During this year, several follow-up clinical examinations have been performed.

Major Findings: Several patients with lesions originally thought to be benign have been reexamined; none has had a change of diagnosis. One patient with a mass lesion that has been regarded as suspicious continues to have no increase of size of the lesion. Two patients who have repeatedly refused to have enucleation continue to show slow growth of the choroidal melanomas without evidence of metastatic disease six and seven years after diagnosis. In both, visual function remains good. One of these patients is now 85 years old; the other is in his 50's.

Significance to Biomedical Research and the Program of the Institute: Continued follow-up information concerning the course of the enucleated patients and the other patients is important because the registry of melanoma patients created by this project serves as an information resource concerning course of disease.

Proposed Course: The intermittent examination of this small group of patients will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00030-09 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Studies of Parameters of Intraocular Pressure		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Douglas E. Gaasterland Other: Carl Kupfer Lessie McCain Roy Milton	M.D. Chief, Section on Glaucoma M.D. Director R.N. Clinical Technician Ph.D. Chief, Section on Biometry	CB NEI NEI CB NEI OBE NEI
COOPERATING UNITS (if any) Normal Volunteer Office, CC, NIH; Pharmaceutical Development Service, CC, NIH; Biomedical and Engineering Instrumentation Branch, DRS, NIH		
LAB/BRANCH Clinical Branch		
SECTION Glaucoma Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.55	PROFESSIONAL: 0.05	OTHER: 0.50
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>In this continuing study of the <u>parameters of intraocular pressure</u>, young and old <u>normal volunteers</u> and patients with <u>glaucoma</u> and <u>ocular hypertension</u> participate. There is interest in determining the actual values of the parameters in eyes not affected by medications and in determining the acute and chronic <u>effects of antiglaucoma medications</u> alone and in combination upon the parameters in normal and in diseased eyes.</p>		

Project Description:

Protocol Number: 75 EI 114

Objectives: To evaluate parameters of intraocular pressure in normal eyes and eyes with ocular hypertension or glaucoma before and after anti-glaucoma medications.

Methods Employed: Replicate studies are done upon experienced human participants. Seven parameters are determined before and after medication: intraocular pressure, episcleral venous pressure, total facility, true facility of outflow, pseudofacility, aqueous flow, and ocular rigidity. Acute drug effects are emphasized. Chronic drug effects are studied by use of the Ocusert system for pilocarpine and in patients receiving monocular treatment in the ocular hypertension protocol of Dr. Ballintine (Project No. Z01 EY 00150-07).

Major Findings: Work upon a masked trial to test the effects of dilute atropine eyedrops upon intraocular pressure in young normal volunteers is nearing completion. Each volunteer has replicate sessions with the three concentrations of atropine or of diluent being given in a symmetric manner--one eye atropine, one eye diluent. Neither the person measuring the pressures nor the volunteer knows which eye has been treated, nor the concentration administered. As soon as the last testing is complete the code identifying the strength of medication and which of the paired bottles contains the diluent will be broken and results tabulated.

We previously observed that acute doses of the beta receptor stimulator isoproterenol, in a 1% solution applied topically, lowered intraocular pressure due to reduced aqueous formation without alteration of total facility, pseudofacility or true facility, in young normal volunteers. Timolol, a beta receptor blocker, in a 0.25% solution applied topically, has nearly identical effects. It causes a marked reduction of intraocular pressure in young normal volunteers. Similar changes are seen after each of these medications given to the eyes of older normal volunteers. In preliminary studies when both these medications are given together to older volunteers, much less effect occurs. This is the topic of a current investigation.

Significance to Biomedical Research and the Program of the Institute: Study of patterns of alteration in the parameters of intraocular pressure caused by glaucoma medications allows clearer understanding of their mechanisms of action. Studies of these parameters more clearly define the difference between normal and abnormal. The measurements can be extrapolated to more basic physiologic functions, yielding insight to the function of the human eye. This information is unique in ophthalmic research.

Proposed Course: The project will be continued, emphasizing medication effects upon parameters.

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications:

Gaasterland DE: Efficacy in glaucoma treatment--the potential of marijuana. Ann Ophthalmol 12:448, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00077-03 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Treatment of Neovascular Glaucoma		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Other: Elmer J. Ballintine M.D. Clinical Director CB NEI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION Glaucoma Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>rubeosis iridis</u> and <u>neovascular glaucoma</u> are being recruited. Those with salvageable vision are invited to join this prospective, randomized study of whether cyclocryotherapy or cyclodiathermy is better for the treatment of this disease. Outcome will be judged by assessing preservation of <u>visual function</u> ; adequate control of <u>intraocular pressure</u> , with or without medications; and control of <u>discomfort</u> . It is estimated that approximately 40 nondiabetic and 40 diabetic patients will be needed for this project.		

Project Description:

Protocol Number: 78 EI 17

Objectives: To determine whether one of two methods for ciliary body ablation, cyclodiathermy or cyclocryotherapy, is better for treatment of neovascular glaucoma.

Methods Employed: Patients who are eligible to join the study, and who consent to participate, are randomly assigned to receive one of the two methods of treatment. Follow-up is aimed at identifying adequacy of treatment and identifying complications.

Major Findings: No new patients have entered the study during FY 1980. Of the four previously treated patients, two who underwent cyclodiathermy have pressure in the teens without glaucoma medications; they have no evidence of rubeosis and no pain. Two who underwent cyclocryotherapy have hypotony, cataract, no rubeosis and no pain. A preliminary conclusion is that the amount of cyclocryotherapy is excessive. The protocol will be modified before additional cyclocryotherapy is performed.

Significance to Biomedical Research and the Program of the Institute: This study has potential for indicating the proper management of these difficult secondary glaucoma patients.

Proposed Course: The study will be continued to allow gathering of additional data.

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00088-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) A Computerized Ophthalmic Citation System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Fred C. Chu Other: David G. Cogan Douglas B. Reingold Deborah H. Young	M.D. M.D. M.A.	Senior Staff Fellow Chief, Neuro-Ophthalmology Section Biologist Television Production Specialist
CB CB CB CB	NEI NEI NEI NEI	
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION Neuro-Ophthalmology Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) We have developed a set of <u>computer</u> programs to <u>cross-index</u> an in-office ophthalmic reference file. Citations can be retrieved within seconds. Lists of references can be generated (1) by specific key-words as sought in reference titles and (2) by authors' names. References can be printed according to specific ready-for-publication formats.		

Project Description:

Objectives: Organizing previously collected ophthalmic citations by subject or any other criterion is tedious. Yet if structured well, such a system in many respects becomes an efficient, in-office diagnostic tool for relating current ophthalmic information to the variety of unusual patients examined in a clinical research environment. Such a system allows virtually instantaneous access to references already available in-office, minimizing the need for repetitious visits to copy references elsewhere.

Methods Employed: Reference citations are sequentially numbered and assigned various subject codes prior to entry into a computer. These codes and all authors' names are used as keys to sort citations by subject and author. The resulting cross-reference lists are then placed on microfiche for use.

The lists are completely updated by generating new microfiche whenever approximately 500 new references are added to the file. Between such updates, the last few hundred references can be cross-indexed and printed as a minor appendix.

The reference data can also be searched for specific words in titles, authors' names and journal names.

Lists of references generated are printed in formats ready-for publication in the various journals for which articles are generally submitted for publication.

Major Findings: As used, the computerized retrieval system is a time-saving tool in the recovery of references collected in the office.

Significance to Biomedical Research and the Program of the Institute: This project gives bibliographic support to the other research programs in the Neuro-Ophthalmology Section.

Proposed Course: This program will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00086-02 CB
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Contributions to Ophthalmic Pathology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: David G. Cogan M.D. Chief, Neuro-Ophthalmology Section CB NEI Other: Toichiro Kuwabara M.D. Chief, Pathology Section LVR NEI Fred C. Chu M.D. Senior Staff Fellow CB NEI Gerald Robison M.D. Geneticist, Cell Biologist LVR NEI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION Neuro-Ophthalmology Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The pathology resource in the Cogan collection has been used to document the recent history of <u>ophthalmic pathology</u> in America and to study <u>radiation</u> effects on the eye.		

Project Description:

Objectives: To learn as much as possible from tissue that might contribute to the understanding of disease in the eyes and visual pathways.

Methods Employed: Selected tissues that are obtained after death or by biopsy during life are subjected to routine microscopic preparation and, where indicated, to special staining and electron microscopy.

Major Findings: Of the several studies done during the past year, the following led to reasonably definitive conclusions or are otherwise noteworthy.

I. Conjunctival biopsies provide a simple and effective means for confirming several of the storage diseases (e.g. ceroid-lipofuscinosis, and Niemann-Pick disease).

II. Gaucher's disease is occasionally accompanied by epiretinal and intraretinal white spots that prove to be collections of turgid histiocytes (Gaucher cells) microscopically. On the other hand the alleged "cherry red spot" in Gaucher's disease could not be confirmed.

III. Several clinical and pathologic studies were made of considerable but limited interest. These included an early case of retrolental fibroplasia, a parachiasmal mass with atypical features of sarcoid, an unusual ciliary body tumor and other cases that happened our way and seemed to warrant special attention.

Significance to Biomedical Research and the Program of the Institute: Progress consists not only in research directed to preprogrammed goals but also in chance observations that arise through serendipity. Unplanned histopathologic observations often suggest new lines of observation.

Proposed Course: To take full advantage of opportunities to study tissue that may become available.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Cogan DG, Chu FC, Gittinger J, Tychsen L: Fundal abnormalities of Gaucher's Disease. Arch Ophthalmol (in press).

Cogan DG: Aging and the eye. Introduction and general comments, in Hands SS (ed): Biology of Special Senses in Aging. Michigan, Institute of Gerontology University, 1979, pp 43-78.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00091-02 CB									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less) Disorders of Vision with Cerebral Disease											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: David G. Cogan</td> <td style="width: 33%;">M.D. Chief Neuro-Ophthalmology Section</td> <td style="width: 33%;">CB NEI</td> </tr> <tr> <td>Other: Fred C. Chu</td> <td>M.D. Senior Staff Fellow</td> <td>CB NEI</td> </tr> <tr> <td>Douglas B. Reingold</td> <td>M.A. Biologist</td> <td>CB NEI</td> </tr> </table>			PI: David G. Cogan	M.D. Chief Neuro-Ophthalmology Section	CB NEI	Other: Fred C. Chu	M.D. Senior Staff Fellow	CB NEI	Douglas B. Reingold	M.A. Biologist	CB NEI
PI: David G. Cogan	M.D. Chief Neuro-Ophthalmology Section	CB NEI									
Other: Fred C. Chu	M.D. Senior Staff Fellow	CB NEI									
Douglas B. Reingold	M.A. Biologist	CB NEI									
COOPERATING UNITS (if any) None											
LAB/BRANCH Clinical Branch											
SECTION Neuro-Ophthalmology Section											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205											
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1									
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Visual disturbances of <u>neurologic disease</u> have been systematically recorded in preparation for diagnostic evaluation.											

Project Description:

Protocol Number: General Consultations

Objectives: To correlate visual symptoms with the site and nature of the lesion in the brain of patients with various neurologic diseases.

Methods Employed: A documentary record is made, by means of video and script recording, of the symptoms experienced by patients with lesions of the brain. These symptoms are correlated with visual field and other neurologic abnormalities and eventually with the nature of the lesion to provide a diagnostic system. It is hoped to submit the data to computer storage.

Major Findings: In addition to the specific analysis of symptoms from non-dominant hemisphere lesions, referred to in the FY 1979 Annual Report and continuing in the present year, particular attention is currently being directed to the neurologic basis for oscillopsia, the significance of binasal hemianopia, and various visual agnosias. Except for the visuo-spatial disorders, no series of symptoms has yet been sufficient to permit definitive conclusions.

Significance to Biomedical Research and the Program of the Institute: Only through a record of a patient's symptoms are we able to provide diagnostic significance to the medical history.

Proposed Course: We will continue to document patients' accounts of their illnesses, and when sufficient evidence is accumulated on any one symptom or group of symptoms, we will subject the data to critical analysis.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Neural Mechanisms)

Publications:

Cogan DG: Visuospatial dysgnosia. Am J Ophthalmol 88:361-368, 1979.

Cogan DG: Some Neuro-ophthalmic syndromes. Trans Pa Acad Ophthalmol Otolaryngol 130, 1979.

Cogan DG: Stroke. Chapter XI. Handbook of Neuro-ophthalmology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00089-02 CB																																				
PERIOD COVERED October 1, 1979 to September 30, 1980																																						
TITLE OF PROJECT (80 characters or less) The Eye and Metabolic Disease																																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">David G. Cogan</td> <td style="width: 10%;">M.D.</td> <td style="width: 40%;">Chief, Neuro-Ophthalmology Section</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Fred C. Chu</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Lawrence Tychsen</td> <td>M.D.</td> <td>Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Toichiro Kuwabara</td> <td>M.D.</td> <td>Chief, Pathology Section</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>W. Gerald Robinson</td> <td>Ph.D.</td> <td>Geneticist, Cell Biologist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>John Barranger</td> <td>M.D.</td> <td>Acting Chief, Clinical Investigation Service</td> <td>DMN</td> <td>NINCDS</td> </tr> </table>			PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI	Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI		Lawrence Tychsen	M.D.	Staff Fellow	CB	NEI		Toichiro Kuwabara	M.D.	Chief, Pathology Section	LVR	NEI		W. Gerald Robinson	Ph.D.	Geneticist, Cell Biologist	LVR	NEI		John Barranger	M.D.	Acting Chief, Clinical Investigation Service	DMN	NINCDS
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	W. Gerald Robinson	Ph.D.	Geneticist, Cell Biologist	LVR	NEI																																	
	John Barranger	M.D.	Acting Chief, Clinical Investigation Service	DMN	NINCDS																																	
COOPERATING UNITS (if any) Development and Metabolic Neurology Branch, NINCDS																																						
LAB/BRANCH Clinical Branch																																						
SECTION Neuro-Ophthalmology Section																																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																						
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2																																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																						
SUMMARY OF WORK (200 words or less - underline keywords) <p>Characteristic dysfunctions of the <u>visual</u> and <u>eye motor systems</u> occur in certain <u>inborn errors of metabolism</u>. The abnormalities presumably stem from the intracellular accumulation of abnormal storage materials, which are cytotoxic.</p>																																						

Project Description:

Objectives: To evaluate the use of ophthalmic abnormalities in the diagnosis and elucidation of inborn errors of metabolism.

Methods Employed: Patients with inborn errors of metabolism referred from Protocol 76N261 are fully examined for visual or ocular motility disturbances. Pertinent findings are photographically recorded or videotaped. The ophthalmic observations in each patient are considered in light of the neurological and biochemical findings. In a number of these patients, conjunctival biopsies have been performed.

Major Findings: In Gaucher's disease, we have noted a spectrum of horizontal gaze disturbances, one form of which simulates congenital ocular motor apraxia. We have studied a number of patients having Niemann-Pick variant, notable for ataxia and foam cells and a characteristic downgaze paralysis. This past year we have added to our collection of pingueculae (numbering now eight) accumulated from patients with Gaucher's disease and, contrary to reports in the literature, do not find Gaucher cells in any of them. In the 60 or so patients we have personally examined with Gaucher's disease, we have looked for the cherry-red spot, as reported in the literature, but have found none.

Significance to Biomedical Research and the Program of the Institute: New observations in the study of inherited disorders add to our understanding of these conditions, especially as clinicopathologic correlations are made.

Proposed Course: This program will be continued since there are continuing referrals of patients with metabolic disorders to the Neuro-Ophthalmology Section.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Chu FC, Kuwabara T, Cogan DG, Schaefer EJ, Brewer HB Jr: Ocular manifestations of Tangier disease. Arch Ophthalmol 97:1926-1928, 1979.

Cogan DG, Chu FC, Gittinger J, Tychsens L: Fundal abnormalities of Gaucher's disease. Arch Ophthalmol (in press).

Cogan DG, Chu FC, Reingold DB, Barranger J: Ocular motor signs in some metabolic diseases. Costenbader Lecture. Annual meeting of the American Academy of Pediatric Ophthalmology. San Diego, California (in press).

Cogan DG, Schulman J, Porter RJ, Mudd HS: Epileptiform eye movements with methylmalonic aciduria and homocystinuria. Case Report. Am J Ophthalmol 90:251, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00020-06 CB																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Ocular Motor Disorders in Human Subjects																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">David G. Cogan</td> <td style="width: 10%;">M.D.</td> <td style="width: 30%;">Chief, Neuro-Ophthalmology Section</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Fred C. Chu</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Dan Milder</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Douglas B. Reingold</td> <td>M.A.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI	Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI		Dan Milder	M.D.	Visiting Scientist	CB	NEI		Douglas B. Reingold	M.A.	Biologist	CB	NEI
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COOPERATING UNITS (if any) None																										
LAB/BRANCH Clinical Branch																										
SECTION Neuro-Ophthalmology Section																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0																								
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) Quantitative measurement of <u>eye movements</u> is fundamental to understanding <u>ocular motor control</u> . Our method of electro-oculography permits vestibular, optokinetic, and saccadic responses to be recorded visually, graphically, and electronically with computerized analysis of the results. Studies in the past year have emphasized the contrasting abnormalities in patients with <u>cerebellar lesions</u> and those with <u>progressive supranuclear palsy</u> . The former involves primarily the visually monitored systems (e.g. <u>pursuit</u>) whereas the latter involves primarily the <u>saccadic system</u> (e.g. volition). An incidental study has also revealed contrasting abnormalities in practice with <u>Gaucher's disease</u> and with a form of <u>Niemann-Pick's disease</u> . The former are significantly accompanied by a disturbance in horizontal movements (at times simulating <u>congenital ocular motor apraxia</u>) whereas the latter are accompanied by disturbances similar to progressive supranuclear palsy. In neither case is the pathologic basis understood.																										

Project Description:

Protocol Number: 77 EI 140

Objectives: Eye movements occur as prominent features of various neurological diseases affecting the central nervous system. Our objective in this project is to provide evidence on the localizing value of eye movement disorders in neuro-ophthalmic diagnosis.

Methods Employed: In selected patients with metabolic or neurological disorders, we have attempted to quantitate the type and degree of ocular motor abnormality.

We record eye movements with electro-oculography or infra-red-oculography. Eye movements are evoked by rotating a subject in a Bárány chair or by projected visual targets. Both chair and visual targets are controlled by computer, while eye position signal is recorded simultaneously online. The data is analyzed for eye movement waveform, velocity, and latency.

Major Findings: We have been processing eye movement data from 56 patients with cerebellar disease to determine the co-occurrence of selected facets of ocular motor disturbance. Preliminary results indicate that saccadic dysmetria is frequently present when there is cogwheel pursuit. Nystagmus in upgaze, but not in downgaze, is frequently present when there is endgaze nystagmus. We have studied two patients with synkinetic oral and extremity movements associated with eyelid closure. The basis for these abnormalities is presumably aberrant structural connections, which would differ from those seen in Marcus-Gunn jaw-winking.

We have also seen a unique patient who experienced illusory movement of his environment without corresponding eye movement defects. His defect may be related to the thalamic lesion which was seen in the CT scan.

We have reviewed our experiences of the associated abnormalities with ocular motor apraxia. They included spinocerebellar degeneration, retinal degeneration, and Gaucher's disease. Although associated abnormalities are not uncommon, the majority of our patients with ocular motor apraxia did not have any associated systemic disorder. One patient followed after 35 years still showed some residual abnormalities in saccadic generation.

Significance to Biomedical Research and the Program of the Institute: Quantitation of ocular motor disturbances by electronic means aids in the diagnosing of lesions within the central nervous system and contributes to the knowledge of how the brain programs information on eye movements.

Proposed Course: The project will be continued. We have accumulated for review videotapes and electrophysiological recordings of more than 200 patients with movement abnormalities. Observations in selected patients will be added to the study.

NEI Research Program: Sensory and Motor Disorders of Vision--
Strabismus and Other Oculomotor Disorders

Publications:

Cogan DG, Chu FC, Reingold DB, Tychsen L: A long-term follow-up of congenital ocular motor apraxia. Case Report. Neuro-ophthalmology (in press).

Chu FC, Cogan DG, Reingold DB: Lid-Triggered Synkineses. Ophthalmology (in press).

Reingold DB, Snyder RA: Illusory movements of the environment without a corresponding eye movement disorder. Neuro-ophthalmology (in press).

Chu FC, Gillin JC: Lid closure Nystagmus. Neuro-ophthalmology (in press).

Cogan DG, Chu FC: Ocular manifestations of myasthenia gravis. Neuro-ophthalmology - 1982 (in press).

Cogan DG, Chu FC, Reingold DB: Notes on congenital ocular motor apraxia. Associated abnormalities. Neuro-ophthalmology (in press).

Cogan DG, Gittinger JW: Atypical ocular bobbing related to phenytoin. Ann Ophthalmol (in press).

Fishman R, Chu F: Episodes in the history of neuro-ophthalmology Video.

Project Description:

Objectives: Although vitamin A has been shown to inhibit keratinization of corneal and various other epithelia, the mechanism by which vitamin A acts to maintain a normal epithelium is not well understood. The purpose of this study is to determine the biochemical basis for the vitamin A mediated changes in corneal epithelium. An additional purpose is to assess the applicability of the inbred corneal dystrophic mouse as a model of human corneal disease.

Methods Employed: Corneas and conjunctival tissue were excised separately and radioactively labeled in organ culture. Vitamin A was either administered to the animal prior to excision or added to the culture medium. The epithelium was then harvested and the epithelial glycoconjugates separated and characterized by DEAE-cellulose chromatography, molecular sieve chromatography and gel-electrophoresis. Vitamin A therapeutic trials were conducted in a double masked controlled fashion utilizing topical and systemic routes of retinoid administration. Clinical observations were recorded, documented photographically and further confirmed by histologic examination.

Major Findings: Vitamin A stimulates ^3H glucosamine incorporation into a glycoprotein synthesized by corneal and conjunctival epithelium. The molecular weight of this glycoprotein can be estimated at $0.5 - 1.0 \times 10^6$. Analysis of the glycopeptides derived from this glycoprotein showed that ^3H glucosamine incorporation was stimulated in only one of the four glycopeptide types. ^{14}C leucine labeling of the glycoprotein was unchanged. These findings suggest that, in affecting epithelial differentiation, vitamin A alters the glycosylation of this glycoprotein.

Ultrastructural examination of the inbred dystrophic mouse corneas revealed a thickening and irregularity of the corneal epithelial basement membrane, and a hypercellularity of the stroma. Vitamin A administration had no consistent effect in reducing corneal keratinization, ulceration, or in vitro elaboration of collagenase. Biochemically, a stimulation of synthesis of certain glycoproteins was detectable.

Significance to Biomedical Research and the Program of the Institute: Xerophthalmia, which can progress to keratomalacia, is a human corneal disease which is thought to arise, in part, from vitamin A deficiency. This disease involves the keratinization of the corneal epithelium and can lead to blindness. The knowledge gained from this study is expected to indicate the biochemical processes of epithelial differentiation that are directly regulated by vitamin A and thereby permit more effective use of vitamin A as a therapeutic agent. Furthermore, this approach may allow the development of diagnostic procedures that will be useful in clinically evaluating human epithelial diseases.

Proposed Course: We will continue to attempt to determine the functional role of the glycoproteins stimulated by vitamin A in inhibiting or otherwise influencing corneal keratinization and the mechanism(s) by which vitamin A regulates corneal glycoprotein synthesis. The special requirements of the

inbred dystrophic mouse cornea for vitamin A and the differences between its keratinization and human xerophthalmia will be sought.

NEI Research Program: Corneal Diseases--Dry Eyes and Tear Abnormalities, Epithelial Disorders, and Drug Delivery

Publications:

Hassell JR, Newsome DA, DeLuca LM: Increased biosynthesis of specific glycoconjugates in rat corneal epithelium following treatment with vitamin A. Invest. Ophthalmol Vis Sci 19:642-647, 1980.

Hassell JR, Newsome DA: Vitamin A induced alterations in corneal and conjunctival epithelial glycoprotein biosynthesis. Ann NY Acad Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00057-02 CB																																																
PERIOD COVERED October 1, 1979, to September 30, 1980																																																		
TITLE OF PROJECT (80 characters or less) Ocular Connective Tissue, Macromolecules and their Function in Vision																																																		
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SUMMARY OF WORK (200 words or less - underline keywords) The structure and function of the major connective tissue macromolecules, the <u>collagens</u> and glycoconjugates, are being examined in <u>animal models</u> and <u>human diseases</u> . The extracellular <u>matrix</u> macromolecules present in <u>cornea</u> , <u>sclera</u> , <u>trabecular meshwork</u> , <u>vitreous</u> , and <u>choroid</u> are fractionated and characterized directly using <u>biochemical</u> procedures. Alternatively, samples of these tissues are radiolabeled in organ culture, or cells derived from these tissues are grown and labeled in <u>cell culture</u> . The macromolecular products synthesized by these cultures are similarly characterized. Alterations in the normal composition of collagens, glycoproteins and <u>proteoglycans</u> may accompany or even be the basis for certain visually disabling ocular diseases.																																																		

Project Description:

Objectives: The extracellular matrix of connective tissues consists of an orderly network of collagen fibers, proteoglycans and glycoproteins. The presence, interaction, and arrangement of these structural macromolecules is crucial to the normal function of these tissues, such as the optical clarity of the cornea and the outflow rate of the trabecular meshwork. The purposes of this study include characterization of the collagens, proteoglycans, and glycoproteins normally present in the cornea, sclera, trabecular meshwork vitreous and choroid and the determination of the alterations that occur in these macromolecules in certain ocular diseases.

Methods Employed: Either ocular connective tissue samples will be radio-labeled in organ culture or cells derived from these tissues are grown and labeled in cell culture. The naturally occurring macromolecules are also extracted and characterized. Biosynthetically labeled as well as unlabeled matrix components are characterized using molecular sieve chromatography, DEAE-cellulose chromatography, CMC-cellulose chromatography, and gel electrophoresis, cesium chloride density gradient centrifugation, as well as with specific enzymes, such as collagenase, chondroitinase, keratanase, glycosidases, papain, and pepsin. Chemical characterizations, in terms of amino acid and carbohydrate analysis, are also conducted.

Major Findings: The keratan sulfate proteoglycan and the chondroitin sulfate proteoglycan, the two major proteoglycans of the corneal stroma, were isolated from monkey corneas and characterized. Normal human corneas also contain these two proteoglycans. However, corneas from patients with corneal macular dystrophy contain only the chondroitin sulfate proteoglycan and not the keratan sulfate proteoglycan. Macular dystrophy corneas do contain an unusual glycoprotein not detected in normal corneas. Stromocytes from macular corneas synthesize the normal proportion of collagen types. The unusual glycoprotein in macular corneas may represent the accumulated material which the corneal clouding.

Keratoconus corneas in vitro elaborate a collagenase which is preferentially active against basement membrane (type IV) collagen. This is the second known enzyme with this activity (the other is from a mammary carcinoma). The presence of this enzyme in actual fresh keratoconus tissues was documented by a positive immunofluorescent reaction with specific antibodies.

Trabecular meshwork from rhesus monkeys in organ culture synthesized an unusually large amount of hyaluronic acid. These results are being compared with those from cultures of normal and glaucomatous human trabeculum.

The major monkey and human vitreous proteins are glycoproteins, with hyaluronic acid and collagens comprising proportionately minor constituents. Adding guanidine extracts of bovine cornea to cultures of corneal stromocytes appear to promote the synthesis of a more normal complement of glycoconjugates.

A heparan sulfate proteoglycan was isolated from a murine tumor which elaborates large amounts of basement membrane material. Antibodies against this proteoglycan react with basement membrane structures in the eye.

Significance to Biomedical Research and the Program of the Institute: Connective tissue is by far the predominant tissue of the eye. It is likely that alterations in the quantity or quality of the macromolecules which comprise these tissues will be the basis of certain blinding and visually disabling ocular diseases.

Proposed Course: This study may provide information that will allow the formulation of testable therapeutic modalities. The project will continue by utilizing appropriate animal models and human material. Antibodies to purified glycoconjugates will be prepared for use in clinical and biomedical research.

NEI Research Program: Corneal Diseases---Corneal Transplantation and Stromal Drying and Repair

Publications:

Hassell JR, Robey PG, Barrach H, Wilczek J, Rennard SI, Martin GR: Isolation of a heparan sulfate-containing proteoglycan from basement membrane. Proc Natl Acad Sci USA 77:0000-0000, 1980.

Hassell JR, Newsome DA, Krachmer JH, Rodrigues MM: Macular corneal dystrophy: Failure to synthesize a mature keratan sulfate proteoglycan. Proc Natl Acad Sci USA 77:0000-0000, 1980.

Hassell JR, Newsome DA, Martin GR: Isolation and characterization of the proteoglycans and collagens synthesized by cells in culture. Vis Res (in press).

Rodrigues MM, Krachmer JH, Miller SD, Newsome DA: Posterior corneal crystalline deposits in benign monoclonal gammopathy. Arch Ophthalmol (in press).

Rodrigues MM, Sun T-T, Krachmer JH, Newsome DA: Epithelialization of the corneal endothelium in posterior polymorphous dystrophy. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRANURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00135-08 CB																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Biochemistry of Retina and Pigmented Epithelium in Health and Disease																										
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<p>Investigations are being conducted into the <u>biochemical composition</u> of the <u>sensory retina</u>, <u>pigmented epithelium</u> and <u>choroid</u> in normal and disease states, particularly in <u>animal models</u> of <u>human retinal deterioration</u> and diseased human ocular tissues. The tissue specific distributions of <u>inorganic constituents</u> is studied by flameless atomic absorption, with concentrations of <u>Ca</u>, <u>Cu</u> and <u>Zn</u> of particular interest. The effects of nutrition and <u>genetic background</u> on the progress of chorioretinal deterioration and cataract formation in the retinal dystrophic <u>pigmented RCS rat</u> are under study. <u>Diabetic rodents</u> are being followed for the development of retinal disease that may model human <u>diabetic retinopathy</u>.</p>																										

Project Description:

Objectives: To study the biochemical composition of retinal photoreceptor, neuronal, glial, and pigment epithelial cells in health and disease, and to explore possibilities for prevention or therapy of retinal and/or choroidal disease when a biochemical abnormality has been identified. Diseases in which pigmented epithelium (PE) is involved are of particular interest.

Methods Employed: Twenty-four-hour urine samples from humans with retinal disease are examined for trace metal content. Defined diets are prepared and fed to affected and congenic unaffected retinal dystrophic animals in controlled experiments. Clinical findings are recorded after biomicroscopic and ophthalmoscopic examination and are documented by fundus photography. Analytical methods include flameless atomic absorption spectroscopy, light and electron microscopy, enzymatic assays and standard quantitative biochemical determinations as appropriate.

Major Findings:I. Trace elements in 24-hour urine specimens from patients with pigmentary retinal degenerations:

This study began as an investigation of a report of Gahlot et al. (1976) that the amount of Cu excreted in a 24-hour urine specimen from patients with primary retinitis pigmentosa (RP) was six-fold normal. Our early work did not confirm this finding, as all patient values were within 0-30 μg Cu/24 hours, the range reported for normals. Our subsequent analyses, as well as those of two other groups (U.S. and England), have continued to show Cu values within this range. Specimens of 24-hour urine from a combined total of 170 patients and normal controls have been assayed. The patient group comprised a variety of types of retinal degeneration. Under the masked protocol, the correlation of clinical findings and biochemistry is not done until after the analyses have been performed. The possibility that significant differences among the clinical groups and normals may occur within the 0-30 μg range will be explored. U.S. studies have suggested that low dietary Cu may be more common than realized and a comparison with the Indian diet consumed by the RP patients of Gahlot et al. will be needed.

We have been determining Zn in the same urines in which Cu was being studied. A total of 114 patient urines have so far been analyzed for Zn. The normals ranged from 94 to 765 μg Zn/24 hours, with a mean of 625 ± 84 (SE) for males and 367 ± 70 for females, in agreement with the literature. Among the male group of patients and relatives analyzed so far, 17 have shown abnormally high values, greater than 800 (804-1715 μg /24 hours); some tendency to clustering within families was seen. Among the female group of patients and relatives so far analyzed, 6 have shown values around 700 μg or above (696-1322 μg Zn/24 hours); four of these were related to males showing high Zn excretion, in two families. Some of the patients in the study are children, and controls of the same age and sex will be used in their evaluation; more than 175 patients and controls will be recruited for the study.

Information on body weight, dietary pattern, possible Zn supplements and other pharmacologic or alcohol intake is being collected. Females on estrogen medications may have low values (less than 100 μg Zn/24 hours) when dietary Zn is low; this occurs because estrogens have an anabolic effect, so that Zn is conserved and not excreted in the urine, and with a normal Zn intake of 12.5 mg per day, urinary Zn is not affected. Dietary Zn below the recommended daily allowance may occur not infrequently in the U.S.

II. Dietary factors improving reproduction, growth, and health in RCS and congenic control rats:

Congenic control and dystrophic strains of the RCS rat model of hereditary retinal degeneration bred poorly when they were fed standard laboratory rodent diet (NIH-07) and housed in conventional animal rooms unshielded from pathologic influences. More prolific reproduction and improved growth were obtained with a diet containing higher concentrations of fat soluble vitamins E and A and of fat, and of zinc. An unpasteurized commercial rodent ration (Charles River R-M-H 3500) was supplemented with sunflower seeds to achieve this improvement. Sunflower seed kernels contain 47% fat and abundant linoleic acid (75% of the unsaturated fatty acids). The seeds are highly acceptable to both young and adult rat. On this diet, in contrast to performance on unsupplemented diets, litters of 8 to 11 or more pups occurred as well as calm temperament of dams, excellent milk production, and rapid growth of pups to weaning and beyond. For the first time, this has enabled us to produce young of specified ages for developmental biochemical studies, including ideal control animals.

Fundus observations have been carried out on pink-eyed and black-eyed dystrophic and control rats aged 24 days to 17 months. The dystrophic and control rats can easily be distinguished by this means and effects of therapeutic measures assessed. Animals on the supplemented diet have not had cataracts, although previously 24% of tan-hooded pink-eyed dystrophics and 3% of black-hooded black-eyed dystrophics were reported to show cataracts between 2.5 to 11 months of age (Lavail et al., 1975). Fundus photographs have been made of the dystrophic and control groups with pigmented eyes. The pictures as compared with those previously published, appear to indicate a slower progress of the disease in the animals on the supplemented diet. These clinical observations will be expanded in larger numbers of animals at successive ages and correlated with histopathology.

Commercial laboratory analyses of sunflower seed kernels for mineral constituents are in progress. Controlled breeding experiments using NIH-07 with or without sunflower seeds and Charles River R-M-H with or without sunflower seeds are being conducted in an identical laboratory environment. An attempt is being made to determine more precisely the factor or factors of greatest importance in the success of the supplemented diet so that a single pellet diet may be developed.

III. Models of diabetic retinopathy:

Rodent and primate models of human diabetes mellitus using genetic, chemical and possible viral induction of disease are being evaluated ophthal-

moscopically, fluorescein angiographically and histologically in an attempt to discover a good model for further studies of nonproliferative and proliferative diabetic retinopathy.

Significance to Biomedical Research and the Program of the Institute:

Retinal deteriorations are the major cause of untreatable legal or worse blindness in the United States and probably, taken as an aggregate, in the world. Nutritional and genetic factors are thought to play key roles in human diseases, and can often be studied in detail in animal models. The retinal pigmented epithelium is becoming increasingly appreciated as the primary site of many of these disease processes. Information gained from these studies should contribute to our understanding of human diseases and to initiating and conducting trials of possible therapeutic measures.

Proposed Course: The project will be continued with emphasis on controlled trials of nutritional regimens and rigorous elucidation of nutritional variables in retinal dystrophic and diabetic animals. Human specimens will be analyzed as they become available.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Hess HH, Newsome DA: Supplemented diet improves reproduction and growth of RCS rats with hereditary retinal degeneration and their congenic controls. Lab Anim Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00097-02 CB																		
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TITLE OF PROJECT (80 characters or less) Biochemistry and Biology of Normal and Pathologic Vitreal and Retinochoroidal Tissues																				
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SUMMARY OF WORK (200 words or less - underline keywords) Investigations are being conducted into aspects of specialization of the <u>primate and human retinochoroidal complex</u> with emphasis on the pigmented epithelium. Using a cell culture system, the <u>mitotic potential</u> of <u>human pigmented epithelial cells</u> as a function of donor <u>age</u> , pigmented epithelial cell surface molecules, and in vitro enzymatic activity including <u>tyrosinase</u> are being studied. <u>Fluorescent antibody reactions</u> of fresh human eye tissues and cultured pigmented epithelial and <u>choroidal cells</u> with monospecific antibodies to purified <u>extra-cellular matrix proteins</u> are being compared in normal and pathologic tissues. In vitro characteristics of cellular outgrowth from <u>pathologic human vitreous bands</u> are being documented by light and electron microscopy. The role of various ocular tissue cells in the production of these bands and in <u>traction retinal disturbances</u> has been documented.																				

Project Description:

Objectives: Although the functional and anatomical specialization of the macula versus peripheral retinochoroidal tissues is well-known and easily observed, the cellular and tissue mechanisms which provide for this specialization are not well understood. A major goal of this project is to describe the special biologic capabilities of the retinal pigmented epithelial cell which contribute to cell-tissue function and to responses to disease states including the formation of abnormal tissues in the vitreous cavity and on the retinal surface. We also wish to investigate, using a variety of techniques, possible alterations in normal enzyme systems such as tyrosinase and in extracellular matrix-basement membrane synthesis which may explain disease processes. A necessary adjunct goal is to develop a defined culture system for retinochoroidal cells, especially the pigmented epithelium, which will make possible experiments which cannot be accurately conducted in serum-containing media.

Methods Employed: Fresh and cultured pigmented epithelial, choroidal and, in some cases, retinal cells are harvested enzymatically and maintained in culture media with varying amounts and types of hormonal and nutrient additives. Growth rate, morphology and enzymatic activity are studied in an attempt to determine the defined medium which supports the most differentiated growth. Synthesis of particular extracellular matrix proteins is determined by a modified direct immunofluorescent reaction with monospecific antibodies. Other techniques include a radioactive water release assay for tyrosinase, microdissection, photomicrography with phase and epifluorescent illumination, scanning and transmission electron microscopy, culture on various substrates including collagen heat gels and reaction of cells with sensitized sheep erythrocytes.

Major Findings: Cellular outgrowths from cultured abnormal vitreous bands removed at surgery from patients' eyes with massive periretinal proliferation included at least four presumptive cell types: 1) pigmented epithelially derived, 2) retinal glial, 3) peripheral blood-tissue macrophage, and 4) fibroblastic cells. Observations of control and cell-seeded collagen heat gels in vitro revealed that only those gels with cells containing contractile filaments exhibited contraction. This finding supports the notion that traction in the diseased vitreous cavity depends upon cellular capabilities, not properties of the acellular portion of vitreous bands.

Choroidal cells have proved more adaptable to hormone supplemented defined media than pigmented epithelial cells. By weaning pigmented epithelial cells slowly from serum-containing to defined media limited survival can be achieved at present. Fluorescent antibody reactions with antibodies to a variety of extracellular matrix (collagenous and non-collagenous) portions revealed that cultured choroidal cells synthesize and deposit fibronectin whereas pigmented epithelial cells do not. Choroidal cells also deposit significantly more type I collagen in vitro than do pigmented epithelial cells. These differences can help distinguish these cells in vitro despite the fact that their phase contrast morphologies may be quite similar.

Freshly harvested, or more usually, cultured, pigmented epithelial cells from normal or age-matched, rod-cone dysplastic Irish setter pups were exposed to controlled, unsensitized sheep erythrocytes and sheep erythrocytes sensitized with immunoglobulin G. Positive binding was assessed by quantitating rosette formation and revealed that a significant number of pigmented epithelial cells from normal animals formed Ig G-dependent rosettes. This binding was trypsin insensitive. Dysplastic dog cells had three to fourfold lower binding than those cells from normal animals. Cultured normal conjunctival fibroblasts, not known to be phagocytic in vivo, showed no binding. Neither pigmented epithelial cell type formed rosettes with unsensitized erythrocytes. The maximal binding observed with pigmented epithelial cells was approximately one-fourth that observed with peripheral blood monocytes from normal or dysplastic animals.

Significance to Biomedical Research and the Program of the Institute:
The macula region falls victim to a variety of blinding disease processes which seem to have a predilection for this specialized central retinal area. Knowledge gained about cellular and molecular mechanisms which provide for the specialization of this fine-vision area is crucial, not only to our understanding of the normal functioning of the macula, but also to pathological processes. Indeed, certain biochemical alterations in animal models of human retinal disease have indicated that cyclic nucleotide levels may even be casually related to certain types of retinal degenerative disease. The techniques described aid in creating an in vitro system in which metabolic functions of normal and pathologic pigmented epithelial and choroidal cells can be accurately studied and manipulated. By coupling morphology with immunologic techniques, it is increasingly possible to identify pathologic cells of vitreous bands, epiretinal and macular fibrosis, all important causes of severe visual impairment or blindness. Increased understanding of the pathologic mechanisms and cell sources should aid in devising improved methods of treating several serious disorders.

Surface properties of specialized phagocytes, as well as the particles they anteriorize, influence the regulation of phagocytosis. Monocytes and other leucocytes from several species have been shown to possess immunoglobulin and complement-dependent surface receptors. Our demonstration that canine retinal pigmented epithelial cells also bind Ig G-sensitized particles and that this binding is altered in cells from animals with rod-cone dysplasia indicates that certain immune systems may play an important role in regulating pigmented epithelium phagocytosis of shed photoreceptor outer segment discs. Because abnormalities of photoreceptor-renewal processes have been shown to play a role in certain animal models of human disease, a greater understanding of the possible role of the immune system in these critical processes could be crucial to advancing our understanding of and developing possible treatments for various degenerative processes.

Proposed Course: Studies to investigate the sources and methods of turnover and renewal of Bruch's membrane will be continued and expanded. Additional emphasis will be placed on adding quantitative determinations to the qualitative immunofluorescent studies and on alterations in the retinochoroidal

complex with aging. Investigations of the interphotoreceptor matrix will be initiated. It is expected that these investigations will be crucial to our understanding of the functioning of the pigmented epithelium as a metabolically active support layer for photoreceptors, of the pigmented epithelial-Bruch's membrane selective barrier for nutrients and other vital factors, and of the responses of these cells to various disease processes.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Newsome DA, Fletcher RT, Chader GJ: Human retinal cyclic nucleotides vary by area. Invest Ophthalmol Vis Sci (in press).

Newsome DA, Rodrigues MM, Machemer RM: Human massive periretinal proliferation: In vitro characteristics of cellular components. Arch Ophthalmol (in press).

Spencer R, Newsome DA, Schepens CL: Limited superficial debridement improves corneal clarity during closed vitrectomy. Am J Ophthalmol 89(1): 137-138, 1980.

Newsome DA, Rahe AE, Silver K: Pigmented epithelial cells in culture deposit a distinctive group of extracellular matrix proteins. IV International symposium on the structure of the eye, Guadalajara, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00098-02 CB																																										
PERIOD COVERED October 1, 1979, to September 30, 1980																																												
TITLE OF PROJECT (80 characters or less) Clinical and Laboratory Studies in Macular and Tapetoretinal Degenerations																																												
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INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																												
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SUMMARY OF WORK (200 words or less - underline keywords) Clinical investigations are underway to determine the early <u>natural history</u> of <u>senile macular degeneration</u> . <u>Drusen</u> and <u>serous and hemorrhagic detachments</u> of the retina and retinal pigmented epithelium, as well as <u>choroidal neovascularization</u> , are considered manifestations of senile macular degeneration and will be studied by serial recordings of the anatomical appearance and visual functioning of eyes at high risk of developing disease. These results will be compared with those obtained from fellow eyes with more advanced disease, age <u>matched normals</u> , and those with other maculopathies including that of <u>retinitis pigmentosa</u> . Serum, and in some cases, 24 hour urine, levels of hormones including <u>melatonin</u> , <u>cortisol</u> , <u>thyroid</u> and <u>trace metal ions</u> are being determined. By emphasizing studies of members of affected family clusters and using various genetic markers such as <u>HLA antigens</u> to segregate the affected from the unaffected members, it is hoped that those factors materially associated with the appearance and/or progression of various <u>retinal degenerative conditions</u> will be elucidated.																																												

Project Description:

Protocol Number: 79 EI 16, 79 M 117 (NIMH)

Objectives: Senile macular degeneration has been extensively studied in its more advanced forms and its devastating effects on vision are well-known. However, little is known of the early natural history of the disease, particularly the relationship of anatomical findings such as drusen and retinal pigmented epithelial detachments to the presence and progress of the disease. It has also been suggested, but not well substantiated, that senile macular degeneration is a dominantly inherited form of retinal dystrophy, casting doubt on the theory that the pathogenesis of the disease is simply linked to degenerative changes associated with aging. A variety of studies have been published which indicate that alterations in the metabolism of copper in the body has a significant effect on pigmentary and perhaps other retinal degenerations. Other published experimental evidence has indicated a significant influence of various hormones, such as thyroid hormones, on the integrity and differentiation of retinal pigmented epithelial cells in vitro. This project was designed to bring multiple disciplines to bear in a broad-scale investigation of this complex family of retinal degenerative disease processes, with particular emphasis on understanding the sites at which the disease processes are initiated, the interrelationship of the various tissues in the retino-choroidal complex as the disease advances, and learning more about the possible genetic linkage of senile macular disease and other retinal deteriorations.

Methods Employed: Patients are recruited into the study upon referral from an ophthalmologist, and are admitted into the study according to the NEI protocol (79 EI 16) or for melatonin studies under the NIMH protocol (79 M 117). Volunteers are obtained for use as age matched controls through the Clinical Center volunteer office. Patients, after giving informed consent to participate in the study, provide a complete history and receive a routine ophthalmic examination and fundus photography complemented with blood and urine studies, genetic typing, psychophysical and electrophysiological studies, and fluorescein angiography when indicated. Patients are followed at regular intervals to evaluate changes, if any, with time. Twenty four-hour melatonin studies are done on overnight admission. The hormone levels are analyzed by mass spectrometry and radioimmunoassay.

Major Findings: A significant number of patients with pigmentary tapeto-retinal degeneration have been found to have a cluster of elevated serum values of various hormones and trace metals. These alterations seem to run in a distinct pattern within certain families that can be segregated according to their genetic typing. In the course of the study, a family with a previously undescribed type of pigmentary macular retinal degeneration was discovered and thoroughly evaluated. These findings shed further light on the progression of changes in the retinal vasculature and changes in the retinal pigmented epithelium layer in this disease. The 24-hour urinary excretion of zinc was found to be elevated in certain patients with retinitis pigmentosa, but not in others. Twenty-four hour urinary copper excretions have been abnormal in

certain patients, but since this study is being done in a masked fashion and is not complete, the correlation with disease is not now known.

Carrier proteins of trace metal ions, especially ceruloplasmin, are being studied in detail. Studies to quantify unbound and bound protein and metal components are underway.

A retrospective review of color visual function test results, visual acuity, corneal and fundus appearance and dosage of chloroquine and hydroxychloroquine in 150 patients seen in NIAMDD clinics was conducted. Prevalence in this population of elevated cone thresholds was 13/150, of definite retinopathy 6/150, of definite corneal lipidosis 2/150 and of possible retinopathy 6/150. All cases of toxicity occurred at total doses above 200 grams. Possible differences in toxicity of chloroquine and hydroxychloroquine are being studied.

Melatonin rhythms are controlled by bright visible light in human beings. The usual diurnal variation (melatonin secretion is shut off in daylight) which is entrained by sunlight appears to be free-running in patients with severe visual impairment from retinal deterioration.

Significance to Biomedical Research and the Program of the Institute:

Senile macular disease is the leading cause of legal blindness in the United States and the United Kingdom and perhaps more frequently, the cause of disability or impairment less severe than legal blindness. There is no effective treatment at present for this disease nor is there for all tapeto-retinal degenerations, which, taken as an aggregate, form the leading cause of untreatable blindness in the world. Some of the processes which appear to constitute the disease picture, such as neovascularization, do occur in other parts of the body. Knowledge gained from this study should be instrumental in understanding the progression and the possible inheritance of these diseases and should contribute to devising studies of more effective modes of treatment. In addition, basic facts which may be learned about certain processes involved in these diseases could have wide applicability to various tissues and organ systems in the body. Melatonin may be the "master" hormonal regulator of human biological clocks, and may be involved in renewal systems in the retina.

Proposed Course: Patients will continue to be recruited into this study during the coming year and will be evaluated thoroughly and followed for a period of three to five years. The recruitment goal is 100 patients. Because of positive findings in the clinical studies, laboratory experiments to evaluate the importance of these observations and their possible effect on ocular tissues will be performed both with animals and with cultured cells. By combining a clinical and laboratory approach, it may be possible to learn information which will point to possible therapeutic trials. Special attention will be given to improving the collection of pathological human material for study and to expanding the already active tissue donor program among the patients under study here.

NEI Research Program: Retinal and Choroidal Diseases--Macular Diseases

Publications:

Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP: Light Suppresses melatonin secretion in man. Science (in press).

Laboratory of Sensorimotor Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1979 - September 30, 1980

REPORT OF THE CHIEF, LABORATORY OF SENSORIMOTOR RESEARCH
Robert H. Wurtz, Ph.D.

Research during the year covered by this second annual report of the Laboratory of Sensorimotor Research concentrated on the visual and oculomotor systems within the primate brain. Experiments ranged from those analyzing the visual processing which leads to perception to those concentrated on the initiation and guidance of eye movements. Since our goal is an understanding of the visual and oculomotor functions of man, all these experiments were performed on rhesus monkeys, whose visual and oculomotor behavior is remarkably similar to that of man in many respects.

Visual information reaches the brain from the eye by either of two major pathways, one through the thalamus (the lateral geniculate nucleus) to the striate area of the occipital lobe of cerebral cortex, the other directly to the midbrain which includes the superior colliculus. This second visual pathway also carries visual information from the superior colliculus through the pulvinar nucleus of the thalamus to the prestriate visual areas of the occipital, parietal, and temporal lobes of cortex that are essential to visual perception. Three types of cells have been found in the pulvinar nucleus: pan-directional cells which respond to stimuli moving across the visual receptive field in any direction; directionally selective cells which respond to stimuli moving only in certain directions; oriented cells which discharge to stimuli moving in one direction or occasionally in two opposite directions. Thus, the type of visual information transmitted by cells in the pulvinar nucleus overlaps that found in cells of the superior colliculus but contains more information about the orientation and direction of movement than is generally found in superior colliculus cells.

A possible source of this other visual information is revealed by anatomical experiments which show an orderly projection from the primary visual cortex, the striate cortex, to the inferior and lateral pulvinar. Within the rostral third of the inferior pulvinar, projections from striate cortex show an orderly projection from central to far peripheral vision as one moves from dorsal lateral to ventral medial. A portion of the lateral pulvinar receives a projection from that part of striate cortex representing central vision. Previous studies have shown a retinotopic arrangement of striate and pulvinar projections to a region of prestriate cortex (area OB) and, coupled with these present studies, indicate the existence of two sources of striate input to the prestriate area that are in perfect register; one direct from one cortical area to another cortical area and another indirect via the inferior and lateral pulvinar.

Visual processing in two prestriate areas was studied. In parietal cortex most cells were found to be dependent for their visual response upon the input from striate cortex, but small islands of responsive cells remained following ablation of the striate cortex. In inferior temporal

cortex, cells were identified which required precisely oriented stimuli but whose fields were small, circumscribed, and near the center of the visual field.

In order for normal visual processing to occur, the central area of the retina, the fovea, must be directed from one part of the visual world to another. This is done by rapid or saccadic eye movements which move the eye at high velocities from one position to another. Since these saccadic eye movements are essential for normal vision, it is not surprising that we have found extensive areas of the brain related to this visual-motor control, and most of the work of the laboratory has concentrated on this visual-motor system.

Cells in an area of the cerebral cortex, the frontal eye fields, are clearly involved in this visual-motor process. For over a century this area has been known to be related to the initiation of saccadic eye movements, but previous work in this laboratory has shown that the only cells in this area discharging before saccadic eye movements are cells which respond to the visual stimulus that is the target of such eye movement. These visual cells have now been found to be limited to one area of the frontal eye field and to be organized so that their visual receptive fields form a retinotopic map. Stimulation within this map drives the eye to the same part of the visual field where cells with visual receptive fields can best be stimulated. The stimulation threshold is elevated if the monkey is actively fixating on one target as opposed to looking for another target. These experiments suggest that the contribution of the frontal eye fields to the control of saccadic eye movements is a specification of a visual target that is to be the target of the eye movement. This control is not an obligate command but instead is subject to interaction and interference of other brain areas, for example, those areas involved in visual fixation.

Another region of the brain that may be involved in the initiation of movement is the basal ganglia; damage to this area produces a paucity of movement including a paucity of saccadic eye movements. Investigation of one area of the basal ganglia, the pars reticulata of the substantia nigra, has indicated that this area may be involved in the visual initiation of saccadic eye movements. Cells in this area have a high discharge rate. Some cells show a decrease in discharge rate before the onset of a saccade. Other cells show the decrease in discharge rate to a visual stimulus alone. However, when the visual stimulus is the target for the saccadic eye movement, the decrease in discharge is more pronounced than when the monkey does not use the target. Since the decrease in discharge rate of these cells occurs before the onset of the saccade, they might act to initiate a saccade by releasing a tonic inhibition on those neurons in the superior colliculus that discharge before saccadic eye movements. These experiments suggest that the basal ganglia may act on the oculomotor system via a pathway through the pars reticulata of the substantia nigra and the superior colliculus.

Work on the superior colliculus itself has shown that even complete ablation of the structure does not lead to an inability to make saccades to visual targets as long as this ablation does not extend into adjacent

structures. However, visual detection abilities of the monkey are impaired just after the lesion is made, and there is a persisting visual-oculomotor neglect. This neglect takes the form of fewer saccades to the visual field served by the ablated superior colliculus and a reduced effect of distracting stimuli occurring in that visual field. The invasion of the lesion into the mesodiencephalic junction and posterior-medial thalamus, however, leads to a severe deficit in the ability of the monkey to make saccades to the peripheral visual field. This horizontal gaze palsy seems to be specifically related to more eccentric positions of the eye in the orbit rather than simply to an eye movement in the peripheral part of the retinotopically organized visual field. These observations emphasize the significant role of yet another region of the brain, the posterior thalamus and mesodiencephalic junction, in the control of visually-guided saccades.

In response to damage or other alterations in the demands on the oculomotor system, adaptive mechanisms are responsible for maintaining appropriate performance of the saccadic eye movement system. Previous work indicated that this adaptive mechanism has two components: one which maintains saccadic accuracy and one which suppresses postsaccadic ocular drift--a drift of the eye after the initial rapid movement. Experiments this year have studied the component responsible for postsaccadic ocular drift and have shown that a slip across the retina of a full field visual stimulus is both necessary and sufficient to elicit this adaptation in normal monkeys. The adaptive response was then studied in two monkeys with complete bilateral ablations of the flocculus of the cerebellum since previous experiments had shown that both adaptive components in the saccadic system are dependent upon an intact cerebellum. The results showed that the ability of the brain to respond to the retinal slip following saccades was reduced over tenfold by the cerebellar ablations.

A modulation of visual processing by the saccadic system was noted in both the pulvinar and the superior colliculus. Cells in the pulvinar show a decreased activity following saccadic eye movements even when the eye movements are made in complete darkness. These visual cells are similar to those cells in the superior colliculus which show a suppression following saccades. In the superior colliculus, a test was made of the hypothesis that this suppression results from activity of a type of cell in the frontal cortex which discharges after saccadic eye movements and which projects to the superior colliculus. Since the suppression in the colliculus persists after ablation of frontal cortex, these cells cannot be the source of the suppression. The input responsible for modulating the visual responsiveness of cells in the superior colliculus and pulvinar remains to be determined.

It will be clear from this summary of work on visual processing and saccadic eye movements that the problem in understanding visual-motor behavior cannot be solved by analysis of one localized area within the brain but instead must be approached by determining the areas of the brain involved and what each contributes to the brain circuitry necessary for visual perception or visually-guided eye movements. An understanding of how this neural system within the brain is organized is essential for analyzing the deficits of patients with diseases affecting the visual and oculomotor systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00053-02 LSR																		
PERIOD COVERED October 1, 1979, to September 30, 1980																				
TITLE OF PROJECT (80 characters or less) Modulation of Visual Processing by Saccadic Eye Movements																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">Robert H. Wurtz</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Chief</td> <td style="width: 10%;">LSR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Barry J. Richmond</td> <td>M.D.</td> <td>Medical Officer</td> <td>LN</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Mortimer Mishkin</td> <td>Ph.D.</td> <td>Research Psychologist</td> <td>LN</td> <td>NIMH</td> </tr> </table>			PI:	Robert H. Wurtz	Ph.D.	Chief	LSR	NEI	Other:	Barry J. Richmond	M.D.	Medical Officer	LN	NIMH		Mortimer Mishkin	Ph.D.	Research Psychologist	LN	NIMH
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SUMMARY OF WORK (200 words or less - underline keywords) We have continued our investigation of how cells in the <u>visual pathways</u> in the primate reduce the sensory stimulation resulting from their own rapid or <u>saccadic eye movements</u> . Cells in the <u>superior colliculus</u> of the <u>monkey</u> have previously been shown to receive an input parallel to the occurrence of saccadic eye movements which reduces their response to the stimulation occurring during saccades. One likely source of this <u>corollary discharge</u> is the <u>frontal eye field</u> area of frontal cortex. To test this hypothesis we recorded activity of cells in the superior colliculus of monkeys with intact and ablated frontal cortex. Cells which show the effect of a corollary discharge were encountered just as frequently in monkeys with and without frontal eye field lesions. The frontal cortex cannot, therefore, be the sole source of a corollary discharge to the colliculus of the primate.																				

Project Description:

Objectives: The visual system of both man and monkey should be constantly interrupted by stimulation occurring during the rapid or saccadic eye movements which move the center of gaze from one part of the visual field to another. Experiments in this laboratory have shown that in one of the visual pathways into the brain, that to the superior colliculus in the brainstem, the discharge of some cells is suppressed following saccadic eye movements. This suppression is strong enough to reduce the response of these cells to the visual stimulation occurring during saccades. The suppression is a result of an input from another part of the brain which occurs as a corollary to the saccadic eye movement.

One likely candidate for such a corollary discharge is the frontal eye field area (area 8) of the cerebral cortex. There is a monosynaptic connection from the frontal eye fields to the superior colliculus, some cells in the frontal eye fields discharge after a saccade just at the time the suppression occurs in the colliculus, and stimulation of the frontal eye fields of the cat produces an inhibition of the superior colliculus cells. On theoretical grounds, it has been suggested that frontal cortical areas are not involved in the initiation of movements but in the corollary discharge associated with these movements. For these reasons we investigated whether the frontal eye fields of the monkey are a source of the suppression observed in the superior colliculus. To do this we ablated parts of frontal cortex and then determined whether the suppression of cells in the superior colliculus associated with saccadic eye movements persisted.

Methods Employed: Ablations of frontal cortex, which were done by subpial suction under direct visual inspection, were done under general anesthesia. After at least a month after surgery, single cells were recorded in the superior colliculus of the awake monkey. These cells were studied using the recording techniques standard to the laboratory. We looked for visually-related cells in the superficial layers of the superior colliculus that showed a suppression of discharge rate following saccadic eye movements.

Major Findings: The first of two monkeys sustained a unilateral ablation of the frontal eye fields. In the superior colliculus ipsilateral to the ablated frontal eye fields, we found that 16 percent of the visual cells in the superficial layers showed suppression indicative of the corollary discharge. However, 16 percent of the cells on the side ipsilateral to the normal frontal eye field also showed this suppression. In a second experiment, a bilateral ablation was performed on the frontal cortex of the monkey with the lesion extending beyond area 8 along the principal sulcus. Again, cells in the superficial layers of the superior colliculus showed suppression in association with saccadic eye movements. Therefore, we conclude that the suppression effect in the superior colliculus does not require the presence of the frontal eye fields or indeed even a large part of frontal cortex. Either the suppression does not

originate in frontal cortex or it originates in frontal cortex but other areas can compensate for damage to this area.

Significance to Biomedical Research and the Program of the Institute:

The superior colliculus is an important area for the initiation of saccadic eye movements, and the corollary discharge to this area allows the system to be sensitive to stimulus movement in the environment but less sensitive to the same stimulus movement produced by the monkey's own saccadic eye movement. The current results show that the corollary discharge is not dependent upon the frontal areas of the cerebral cortex and strengthen the argument that such an automatic corollary of eye movement is a subcortical function which is probably independent of cerebral cortical control. Since humans as well as monkeys make saccadic eye movements several times per second, the disruption of visual processing occurs in man as well as monkeys. An understanding of the mechanisms which allow the visual perception and the visual guidance of saccades to function in spite of this disruption is essential to an understanding of normal vision in man.

Proposed Course: The current experiments complete the project as originally envisioned. Subsequent experiments on this topic are dependent on a more complete understanding of the primate visual and oculomotor systems which should result from other projects in the laboratory.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Neural Mechanisms)

Publications:

Judge SJ, Wurtz RH, Richmond BJ: Vision during saccadic eye movements. I. Visual interactions in striate cortex. J Neurophysiol 43:1133-1155, 1980.

Richmond BJ, Wurtz RH: Vision during saccadic eye movements. II. A corollary discharge to monkey superior colliculus. J Neurophysiol 43:1156-1167, 1980.

Wurtz RH, Richmond BJ, Judge SJ: Vision during saccadic eye movements. III. Visual interactions in monkey superior colliculus. J Neurophysiol 43:1168-1181, 1980.

Wurtz RH, Goldberg ME, Robinson DL: Behavioral modulation of visual responses in the monkey: Stimulus selection for attention and movement, in Sprague JM, Epstein AN (eds): Progress in Psychobiology and Physiological Psychology. New York, Academic Press, 1980, vol 9, pp 43-83.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00102-01 LSR												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Role of Substantia Nigra in the Initiation of Eye Movements														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">Robert H. Wurtz</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Chief</td> <td style="width: 15%;">LSR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Okihide Hikosaka</td> <td>M.D., Ph.D.</td> <td>Visiting Scientist</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI:	Robert H. Wurtz	Ph.D.	Chief	LSR	NEI	Other:	Okihide Hikosaka	M.D., Ph.D.	Visiting Scientist	LSR	NEI
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LAB/BRANCH Laboratory of Sensorimotor Research														
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INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
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SUMMARY OF WORK (200 words or less - underline keywords) Cells in the <u>pars reticulata</u> of the <u>substantia nigra</u> in the <u>basal ganglia</u> of <u>monkeys</u> have been studied to determine their relationship to the initiation of <u>visually-guided saccadic eye movements</u> . Some cells are related to the initiation of the saccadic eye movements themselves, others to the visual stimulus which is the target for the saccade. Most visual responses are modulated depending on the use of the visual stimulus by the monkey; the decreased response is greater when the monkey uses the stimulus and is frequently eliminated when the monkey uses another distant stimulus as the target for a saccadic eye movement. These cells may represent the output of the basal ganglia to the oculomotor system. Since the pars reticulata cells project to the <u>superior colliculus</u> , they may act on the oculomotor system by acting on the saccade-related cells in the superior colliculus.														

Project Description:

Objectives: The basal ganglia, including the substantia nigra, are involved in the initiation of movement, including rapid or saccadic eye movements. Recent anatomical evidence has shown that the pars reticulata of the substantia nigra projects monosynaptically to the intermediate layers of the superior colliculus. These superior colliculus cells discharge before the onset of saccadic eye movements. A study of the relation of cells in the substantia nigra to the onset of eye movement, therefore, offers the opportunity both to study how the basal ganglia are related to the initiation of eye movements and to study the neural processing which might precede saccades just one step earlier than that seen in the superior colliculus.

Methods Employed: Cells were studied in awake monkeys trained to fixate on a spot of light and to follow that spot of light from one point to another. This training allowed analysis of the discharge of the cell in relation both to visual stimuli and to saccadic eye movements made to the stimuli. Location of cells within the brain was determined by passing current through the electrode at the time of recording and locating the resulting microlesions on the histological sections at the end of the experiments.

Major Findings: Cells in the substantia nigra generally had a steady high rate of discharge between 40 and 100 spikes per second. Many of these cells (but by no means all) showed a decrease in the rate of discharge before the onset of visually-guided saccadic eye movements. These cells fell into two general categories: those with a decrease in discharge related to the initiation of the saccade itself; those related to the visual target guiding the saccade.

The discharge rate of saccade-related cells usually decreased about 100 msec before the onset of a saccade although for some cells the discharge did not decrease until just at the onset of the saccade, and the decrease lasted for between 40 and 150 msec after the beginning of the saccade. If a visual target came on and the monkey did not make a saccade to the target, these cells showed little change in discharge rate; the saccade must occur for the change in discharge to occur. The pars reticulata cells also had movement fields: an area in the visual field where saccades were accompanied by a change in the discharge rate of the cell. These movement fields tended to be in the contralateral visual field but frequently included up or down movements as well. There was also a gradient of change in discharge in different parts of the movement field; saccades made to one part produced a change in discharge rate that was more vigorous than saccades to other parts.

The discharge rate of visually-related cells decreased after the onset of a spot of light in one part of the visual field, regardless of whether the monkey actually made a saccade to the stimulus or not. The latency for the decrease in discharge usually ranged from 70 to 150 msec, substantially

longer than the shortest latency observed in the superior colliculus (40 msec). Like the movement fields, the visual receptive fields were located in the contralateral visual field and frequently included the vertical meridian. Visual receptive field sizes varied from a few degrees in diameter to nearly a hemifield.

Most of the visual responses were strongly modulated depending upon whether the monkey used the visual stimulus falling in the receptive field as a target for a saccadic eye movement. The modulation occurred both when the monkey used the stimulus falling in the receptive field as a target for a saccade and when he used a stimulus outside the visual receptive field as a target for a saccade. When he used the stimulus falling in the receptive field as a target for a saccade, the decrease in discharge rate was more complete. When the monkey used a stimulus outside the receptive field as a target for the saccade, the response to the stimulus in the receptive field was greatly reduced, frequently showing no decrease in discharge rate.

Significance to Biomedical Research and the Program of the Institute: The substantia nigra of the basal ganglia is suspected to be involved in several disease processes, including Parkinson's disease and Progressive Supranuclear Palsy. In Parkinson's disease the paucity of movement seen with skeletal movements is carried over into a paucity of saccadic eye movements. The current experiments, for the first time, show how the cells in this area in the primate brain discharge in relation to eye movements. Furthermore, the pause in discharge of cells in substantia nigra in relation to eye movements, the known connection of this area to the eye movement related cells in the superior colliculus, permits the development of a hypothesis that it is the pars reticulata of substantia nigra that provides tonic inhibition on cells in the superior colliculus, which after the onset of visual stimulus is reduced permitting the increase in discharge of the cells in the superior colliculus, which in turn contribute to the initiation of saccadic eye movements. The substantia nigra to superior colliculus connections undoubtedly form an important circuit for initiation of saccadic eye movements and an understanding of this circuitry should lead to a more accurate diagnosis of the disease processes that involve this area of the brain.

Proposed Course: The current experiments represent an initial characterization of the reduction of cell discharge in substantia nigra to saccadic eye movements. Work in the next year will concentrate on completing this characterization of the cell discharge and exploring more thoroughly the relation of the cells to the changes in monkey behavior in initiating saccadic eye movements. Stimulation and ablation experiments will be added as well as an attempt to show which cells in the substantia nigra are related to the movement cells in the superior colliculus.

NEI Research Program: Sensory and Motor Disorders of Vision--
Strabismus and Other Oculomotor Disorders

Publications:

Hikosaka O, Wurtz RH: The role of substantia nigra in the initiation of saccadic eye movements, in Fuchs AF, Becker W (eds): Proceedings of the Symposium on the Neural Control of Eye Movements. New York, Elsevier North-Holland (in press).

Wurtz RH, Albano JE, Hikosaka O: Relation of the superior colliculus to the initiation of eye movements. Proc Int Union Physiol Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00055-02 LSR																														
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SUMMARY OF WORK (200 words or less - underline keywords) <p> The role of the <u>superior colliculus</u> and structures in the <u>mesodiencephalic junction</u> in the <u>visual guidance of saccadic eye movements</u> has been investigated. <u>Rhesus monkeys</u> are trained and tested on several tasks that permit measurement and control of visual and oculomotor responses to stimuli in the <u>central and peripheral visual field</u>. After unilateral surgical ablations, the monkeys are retested on the visuomotor tasks in order to assess the effect of the <u>brain damage</u> and to study the course of <u>recovery of function</u>. Immediately after the lesions, <u>visual detection abilities</u> are impaired, but this recovers quickly leaving a longer lasting <u>visual-oculomotor neglect</u>. There is also a loss in the accuracy of visually-guided saccadic eye movements characterized by a <u>conjugate horizontal gaze palsy</u>. This effect appears to be dependent upon damage to more rostral structures rather than to the superior colliculus alone. Future experiments will focus upon localization of relevant structures in the posterior-medial thalamus and mesodiencephalic junction that play such a crucial role in visually-guided saccadic eye movements. </p>																																

Project Description:

Objectives: The superior colliculus, an area on the roof of the midbrain, receives direct retinal projections and projects to important oculomotor centers of the brainstem. Many neurons in this structure fire in response to visual stimuli and also in relation to saccadic eye movements. The initial goal of our research was to examine the contribution of the primate superior colliculus to visual and oculomotor function. In these studies we attempted large lesions of the colliculus in order to study visual and oculomotor behavior in response to central and peripheral portions of the visual field. We found that monkeys with such lesions have a deficit in visual detection that quickly recovers. In addition, we found that they also have a loss in the accuracy of visually-guided saccadic eye movements to peripheral targets, further than about 20° from the fovea. The efforts of the past year have been both to provide a more complete description of the visual and oculomotor deficits and to identify the structures responsible for the deficit.

Methods Employed: Rhesus monkeys are trained preoperatively and tested postoperatively on two sets of computer-controlled tasks. The first set of tasks were designed to study certain sensory aspects of visuomotor behavior. In one of these tasks, the detection task, the monkeys were trained to signal that they had detected the occurrence of a small flashed stimulus (0.2° in diameter) presented at unpredicted locations in the field while maintaining fixation on a central spot of light. Postlesion performance on this task would allow mapping of field deficits throughout $\pm 45^\circ$ of visual angle. In another task, the distraction task, monkeys were trained to respond to the dimming of a central visual stimulus. On occasional trials a peripheral stimulus was flashed; if the monkey broke fixation to acquire the irrelevant unrewarded stimulus, it was considered a distraction. In addition, five minute periods of spontaneous eye movements were recorded pre- and postlesion.

In the second set of tasks, we examined the effect of large colliculus lesions upon saccadic (rapid) eye movements. The monkeys were trained on a saccade task to make an eye movement from a central fixation light to another visual target. The position of the saccade target was varied so that saccades of all directions and amplitudes were studied. In another task, an eccentric eye position task, the monkey made saccades of constant amplitude that were initiated from variable eye positions. Throughout the behavioral training and pre- and postlesion testing sessions, the presentation of visual stimuli was controlled and behavior and eye movement responses were monitored, displayed, and recorded in digital form by computer. Eye movements were previously monitored by using the electro-oculogram. They are currently monitored using the more precise magnetic search coil technique. After prelesion testing the monkeys receive large unilateral surgical ablations of the superior colliculus. This is accomplished by sectioning the corpus callosum and ablating the colliculus by subpial suction. Monkeys are prevented from visual experience during

the postsurgical recovery period preceding the testing period by insertion of translucent contact occluders.

Major Findings: Sensory deficits: Four rhesus monkeys were trained on the detection task. Immediately after surgery, translucent contact occluders were placed into the monkeys eyes to prevent vision during the recovery period. After varying postsurgical recovery periods, the occluders were removed, and they were tested on the detection task. After eight days of recovery, one monkey had a visual field deficit that included central and peripheral portions of the visual field. This deficit quickly recovered during the second postoperative week. Recovery occurred first at more central points and progressed toward the periphery so that by day 13, errors were limited to a region beyond 40° from the fixation point. Other animals tested on day 10, and one on day 13, showed the same pattern and time course of recovery. Therefore, the pattern and time course of this recovery does not depend upon visual pattern experience.

After recovery of the visual detection deficit, we tested the animals on the visual distraction task. We found that the effect of unilateral colliculus lesions was to reduce the number of eye movements to the distracting peripheral stimuli occurring in the visual field contralateral to the lesion. The frequency of eye movements toward the distracting stimulus occurring in the ipsilateral visual field (intact visual field) was not reduced. Similar results were obtained from samples of spontaneous eye movements recorded while the monkeys were free to explore the visual environment between test sessions. There was a greater tendency for the monkeys to make eye movements to positions toward the ipsilateral side than the contralateral side.

Thus, immediately after the lesion there is a sensory loss in the ability to detect visual stimuli, but this deficit quickly recovers. A longer lasting visual-motor deficit seen in the distraction task and in the pattern of spontaneous eye movements can be characterized as a visual neglect of the visual field related to the ablated superior colliculus.

Oculomotor deficits: Three of four monkeys showed a dramatic deficit in the ability to make saccades to targets beyond 20° in the visual field contralateral to the ablated colliculus: saccades fell short of the visual target and were not corrected by a second saccade. This deficit was not due to an inability to detect the visual stimulus since the detection deficit had recovered before these saccade tests were begun. To determine whether the deficit was related to orbital position, the amplitudes of 10° saccades initiated from contralateral and ipsilateral eccentric eye positions were compared. The amplitudes of saccades made in the ipsilateral and central orbital positions were normal but, when the monkey attempted to make saccades from nonprimary eye positions contralateral to the lesion to more eccentric eye positions, the saccades fell short of the target. Thus, regardless of the initial position, the most eccentric eye position which could be attained by these animals was restricted to within about $20-30^\circ$ of primary orbital position. Therefore, this deficit in oculomotor control

was not related to retinotopic stimulus position but instead varied as a function of eccentric orbital position.

Histological results: Reconstructions of the ablations in all four animals revealed that damage to the superior colliculus was complete as intended. However, additional damage, presumably due to an infarct of a vessel overlying the colliculus, caused extensive damage to structures anterior to the colliculus in three animals. In the remaining monkey, this anterior damage was relatively slight. These histological findings correlated with the behavioral results: the three monkeys that could not achieve eccentric eye positions were also those animals with extensive anterior damage; the one monkey without this deficit had these anterior structures largely intact. Several structures in the region of the posterior-medial thalamus and mesodiencephalic junction were involved including the mediodorsal nucleus, internal medullary lamina, the pretectum, the brachium of the superior colliculus, and the posterior commissure.

Significance to Biomedical Research and the Program of the Institute: Experimental animal models of visual-oculomotor disorders provide an opportunity to study in detail the neural substrate and behavioral deficits resulting from central nervous system damage. Initial experiments indicated that large lesions of the superior colliculus produce deficits in the guidance of saccadic eye movements to peripheral visual targets. We have since found that the oculomotor deficits appear to be a function of orbital position. In the neurological literature, similar deficits have been seen in man and are referred to as conjugate gaze palsy. Typically, however, the neurological localization of the lesion was believed to be in the reticular formation, either mesencephalic or pontine. These studies indicate that damage to more rostral areas in the posterior-thalamus and mesodiencephalic junction may also result in gaze palsy.

These experiments also show that immediately following superior colliculus ablations (regardless of involvement of other structures) there is a sensory loss in the ability to detect visual stimuli. This deficit quickly recovers, leaving a longer lasting visual-motor deficit. This visual-motor deficit may represent a form of visual neglect reflecting a loss in a component of visual attention related, though not necessarily limited, to the initiation of eye movements. These present experiments have helped to clarify both the responsible neural structures and the nature of the sensory and motor deficits that might be seen in the clinic.

Proposed Course: Experiments in the next year will attempt to determine whether damage to the superior colliculus in association with other structures in the posterior-medial thalamus and mesodiencephalic junction or, if damage to these structures alone is sufficient to produce the peripheral gaze palsy. These additional studies will add to our knowledge of neural structures and pathways that participate in the generation of eye movements.

NEI Research Program: Sensory and Motor Disorders of Vision--
Strabismus and Other Oculomotor Disorders (Disorders Affecting Control of
Eye Movements)

Publications:

Albano JE, Wurtz RH: The role of the primate superior colliculus,
pretectum, and posterior-medial thalamus in visually-guided eye
movements, in Fuchs AF, Becker W (eds): Proceedings of the Symposium
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Wurtz RH, Albano JE: Visual-motor function of the primate superior
colliculus. Ann Rev Neurosci 3:189-226, 1980.

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SUMMARY OF WORK (200 words or less - underline keywords) Studies are being conducted to determine the mechanisms through which the <u>cerebral cortex</u> exerts controls over <u>eye movements</u> and <u>visual attention</u> in the <u>monkey</u> . <u>Single cell recordings</u> are made while the monkeys perform a series of visual tasks involving eye movements or visual fixation. We have found that the frontal eye fields contain a neural mechanism for the generation of visually-guided eye movements. Visual neurons in this area yield enhanced discharges before eye movements to stimuli in their receptive fields. The amplitude and direction of eye movements evoked by stimulation at the site of frontal visual cells correlate with the receptive field locations for those cells. The threshold and amplitude of eye movements evoked by electrical stimulation of the frontal eye fields can be affected by the act of purposeful visual fixation. When the animal is actively performing a visual task, the brain is relatively refractory to stimulation of the frontal eye fields. These data imply that there is a special neural mechanism involving the frontal fields that is unique to those eye movements involved in purposeful visual behavior.																				

Project Description:

Objectives: Visual attention and the initiation of eye movements are tightly intertwined, yet can be dissociated. The previous work on this project established that the visual neurons in posterior parietal cortex yield an enhanced response to visual stimuli that are relevant to the animal's behavior, independent of the motor nature that the animal might make to that stimulus. This mechanism functions in a powerful way before visually-guided eye movements but functions indistinguishably before other behaviors in which the animal attends to the stimulus without making an eye movement to it. The phenomenon is a general one consistent with neural processes underlying visual attention, but its very generality prevents it from serving as the final cortical pathway for the direction of eye movements. Such a specific cortical mechanism is found in the frontal eye fields, where single neurons give an enhanced discharge in response to stimuli that will be the target for eye movements, but not in association with behaviors that do not involve eye movement. This finding is surprising in view of earlier studies that failed to show a neuronal signal preceding eye movements in the frontal eye fields. However, the signal that we have described occurs exclusively before visually-guided eye movements, and therefore does not occur in association with all eye movements. Since we have identified the frontal eye fields as a cortical center for the control of gaze, it becomes important to know the details of its physiological and anatomical organization. We have therefore begun an intensive survey of neuronal types in the frontal eye fields in order to answer the following questions: what are the neuronal types in the frontal eye fields that relate to eye movement? Are these cells organized in a specific retinotopic or oculomotorotopic manner? What are the efferent connections of this cortical oculomotor area? What are the visual properties of these cells and how do these properties relate to the eye movements evoked by electrical stimulation through the recording microelectrode? If there is a cortical area dedicated to the visual guidance of eye movements, then the neuronal events controlling purposeful visually-guided eye movements must be to some extent different from other eye movements, and the response of the oculomotor system to frontal eye field stimulation should differ depending on whether or not the animal is actively engaged in performing a visual task.

Methods Employed: A digital computer was used for behavioral control, data acquisition, and on- and off-line analysis of monkey behavior, eye movement, and neuronal discharge time patterns. Monkeys were trained on several visuomotor tasks including visual fixation, saccadic eye movements to find a target which may signal a reward, and saccadic eye movements in order to earn a reward for producing an eye movement of the proper amplitude and direction. Activity of single units was measured during these tasks. Stimulation at the site of the recording electrode was performed and the properties of eye movements measured during visual fixation and rest. Eye movements were measured using the magnetic search coil technique so that accurate quantitative measures of eye position and velocity could be obtained. Microlesions were placed at the site of interesting cells and the anatomical locations of these cells were determined in order to reconstruct

the anatomy of the cortical visual area. The anatomical efferents of this area were studied by locating in untrained monkeys the area which had the lowest threshold loci for electrical stimulation and under direct vision placing radioactively labeled amino acids in those low threshold areas in order to determine the neuroanatomical targets of this area.

Major Findings: There are two main classes of cells in the frontal eye fields, visual cells that discharge before the eye movement and nonvisual cells that discharge during and after the movement. The visual cells have large receptive fields with mildly graded responses within their receptive fields. Half of the visual cells give an enhanced response to stimuli in their receptive field when those stimuli are used as targets for eye movements. The cells do not discharge in association with similar eye movements in total darkness. The nonvisual cells discharge during and after eye movements of proper amplitude and direction even in total darkness. Electrical stimulation at the site of the visual eye movement cells results in an eye movement to the most active part of the field and, in general, the threshold for evoking an eye movement is lowest at the sites of visual cells with enhanced responses.

The position of the receptive fields of cells, and the equivalent direction of eye movements evoked by stimulation, form a crude retinotopic map with the upper visual field on the most posterior part of the medial limb of the arcuate sulcus and the lower field appearing to stretch down into the sulcus. Unlike many other visual maps in the brain, the bulk of the area seems to be concerned with the retinal periphery and, if there is a specifically foveal area, it must be quite small. Although there are nonvisual eye movements dispersed throughout this area, the eye movements that best stimulate these cells are not so reliably arrayed as are the visual receptive fields of the visual neurons, nor does the preferred direction of these cells reliably predict the direction of eye movement evoked by electrical stimulation. The traditional frontal eye field is a rather large area encompassing much of the arcuate gyrus. This visual oculomotor area is a much smaller area, with surface dimensions as low as three millimeters by three millimeters.

Stimulation of the frontal eye fields does not induce an absolutely determined eye movement because the eye movements evoked by stimulation interact with the animal's own visual behavior. Thus, if one stimulates the frontal eye field during active visual fixation, the threshold for evoking a saccadic eye movement is higher, and the eye movement is shorter and slower than those evoked from the same site when the animal is at rest and presumably not looking intensely at anything. Detailed quantitative analysis of the eye movements evoked by stimulation during fixation and rest indicates that the shorter saccades are not prematurely truncated longer saccades but actually are programmed to be shorter. This implies that in computing the saccade to be made in conjunction with visual stimulation, the oculomotor system takes active fixation into account and produces a saccade whose dimensions are the weighted sum of the normal eye movement and the fixation signal. Since the fact of visual fixation can alter the

programming of saccades, it may not be that the acts of fixation and eye movement are totally independent but rather that they are a part of a continuum of eye movement control. In this context, the contribution of the frontal eye fields to eye movement control would be the specification of a visual target that is to be the target of an eye movement. This signal is not an obligate command but instead is subject to interaction and interference from other brain areas.

Significance to Biomedical Research and the Program of the Institute: These experiments have demonstrated that there is a small cortical area that is dedicated to the generation of visually-guided eye movements. This area requires a sensory input and uses it to generate a meaningful motor signal. As such, the area may provide a model for sensorimotor integration that is capable of being understood on an extremely sophisticated level since both visual processing by the cerebral cortex and oculomotor dynamic processing by the brainstem are becoming increasingly well understood. In addition, understanding the cortical mechanisms underlying the generation of eye movements can lead to the development of treatment for patients with cerebral lesions which lead to disorders of visuomotor coordination and ocular motility.

Proposed Course: Further exploration of the frontal visual area will be performed in order to correlate the discharge of frontal eye field visual neurons with the various phases of eye movements in order to see if a quantitative model of frontal eye field function can be derived. The preliminary data regarding the retinotopic map of the frontal eye fields will be expanded with special attention paid to the lower field which presumably extends into the arcuate sulcus. Monkeys will be trained in a series of multiple eye movement paradigms to flashed targets in order to see if the discharge of frontal neurons is related to the movement direction or retinotopic position of the target when there is a dissonance between retinotopic and orbital information. The efferents of the adjacent high threshold eye movement areas, whose single neurons do not have visual oculomotor properties, will be studied to see if there is a unique projection system of the visually active area.

NEI Research Program: Sensory and Motor Disorders of Vision--
Strabismus and Other Oculomotor Disorders (Disorders Affecting Control of Eye Movements)

Publications:

Goldberg ME, Bushnell MC: The role of the frontal eye fields in visually-guided eye movements, in Fuchs AF, Becker W (eds): Proceedings of the Symposium on the Neural Control of Eye Movements. New York, Elsevier North-Holland (in press).

Goldberg ME, Robinson DL: Behavioral modulation of visual responses in monkey superior colliculus and cerebral cortex, in Thompson RF,

Hicks LH, Shvyrkov SH (eds): Neural Mechanisms of Goal Directed Behavior and Learning. New York, Academic Press, 1980, pp 397-405.

Goldberg ME: Moving and attending in visual space: Single cell mechanisms in the monkey, in Potegal M (ed): Developmental and Physiological Aspects of Spatial Behavior. New York, Academic Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00047-02 LSR																											
PERIOD COVERED October 1, 1979, to September 30, 1980																													
TITLE OF PROJECT (80 characters or less) Visual Processing in Brains Following Cortical Ablation																													
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																													
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">Michael E. Goldberg</td> <td style="width: 10%;">M.D.</td> <td style="width: 30%;">Research Medical Officer</td> <td style="width: 10%;">LSR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td rowspan="4">Other:</td> <td>M. Catherine Bushnell</td> <td>Ph.D.</td> <td>Guest Worker</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td>Charles J. Bruce</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td>Leslie G. Ungerleider</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td>Mortimer Mishkin</td> <td>Ph.D.</td> <td>Research Psychologist</td> <td>LN</td> <td>NIMH</td> </tr> </table>			PI:	Michael E. Goldberg	M.D.	Research Medical Officer	LSR	NEI	Other:	M. Catherine Bushnell	Ph.D.	Guest Worker	LSR	NEI	Charles J. Bruce	Ph.D.	Staff Fellow	LSR	NEI	Leslie G. Ungerleider	Ph.D.	Senior Staff Fellow	LSR	NEI	Mortimer Mishkin	Ph.D.	Research Psychologist	LN	NIMH
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INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																													
TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.5	OTHER: 0.25																											
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SUMMARY OF WORK (200 words or less - underline keywords) <p> The <u>striate cortex</u> of one hemisphere is removed surgically from rhesus <u>monkeys</u> under direct vision, and the monkeys are allowed to recover from the effects of surgery in a normally lit environment. The monkeys are then trained on a series of tasks requiring <u>visual perception</u> and <u>visually-guided eye movements</u>. They are then prepared for <u>chronic neurophysiological recording</u>, and the activity of <u>single neurons</u> in the <u>posterior parietal cortex</u> both ipsilateral and contralateral to the lesion is studied. Preliminary results indicate that the great bulk of the parietal cortex has its visual responsiveness to stimuli contralateral to the damaged hemisphere eliminated. However, several cells in small areas of the parietal cortex maintain responsiveness to both visual fields. This indicates that visual activity in the posterior parietal cortex is partially dependent upon an intact visual cortex but that some visual information can reach the parietal cortex exclusively through the extrageniculostriate pathways. These islands of maintained visual activity presumably subsume the surprisingly large residual visual capacity found in monkeys and man with damaged striate cortex. </p>																													

Project Description:

Objectives: Information from the retina can reach the parietal cortex in two ways: first, via the lateral geniculate nucleus of the thalamus, then through the striate and prestriate cortex; second, via the superior colliculus, then through posterior thalamic nuclei. These pathways interact: the striate cortex projects to the superior colliculus and the pulvinar of the thalamus, and the pulvinar projects to the prestriate cortex. Nonetheless, these areas are each capable of contributing to visual behavior in the absence of the other. Even in humans, where it was traditionally thought that striate damage led to total visual impairment, it has now become clear that there is some residual visual function in the absence of striate cortex which can be accessed using forced choiced or nonverbal methods of evaluation. Since parietal cortex should receive the subcortical visual pathway in the absence of striate cortex, it was of interest to see if this area had enough visual processing to support the behavior found in the presence of striate lesions. Initial work in this laboratory showed that this residual visual processing could be performed in the cerebral cortex by parietal neurons that are visually responsive even in the absence of striate cortex. This visual activity was limited to a small area of the parietal cortex but within this area seemed quite normal. Recent work on this project has concentrated on a quantitative analysis of the response properties of single neurons in the parietal cortex following striate lesions and a detailed histological localization of the neurons with spared function.

Methods Employed: Rhesus monkeys underwent unilateral striate ablation. After recovery from surgery they were trained to perform a number of visuomotor tasks including visual fixation, visually-guided tracking and saccadic eye movements, visually-guided hand reaching, and response to the flickering of a peripheral stimulus without an eye movement toward the stimulus. Responses of single neurons were recorded in the parietal cortices ipsilateral and contralateral to the striate lesions. The sites of interesting neurons were marked using small electrolytic lesions through the recording microelectrode. The monkeys were perfused with saline and formalin and prepared for histological examination using the celloidin method.

Major Findings: In the parietal cortex contralateral to the lesion, the visually-responsive neurons seemed quite normal except that the representation of the visual field contralateral to the lesion seemed sparser than expected. In the posterior parietal cortex on the same side as the lesion, the representation of the contralateral field (i.e., that field which had no striate contribution) was remarkably limited in extent. In each of two monkeys a visually responsive area was found but it was located in a different place in each. In one, the visually responsive area was located on the posterior bank of the intraparietal sulcus and the adjacent cortical surface. This area appeared quite normal: it had visual responsiveness throughout the visual field, and the behavioral modulation of the visual responses in this area was normal. In the second monkey, a

visually responsive area was found with visual responses limited to the lower visual field, and behavioral modulation of visual responses was absent. This region was in the most posterior part of the parietal cortex, in the superior temporal sulcus. Each area was approximately 3 mm square and was surrounded by larger expanses of cortex with no visual responsiveness to contralateral stimuli.

Significance to Biomedical Research and the Program of the Institute:

Even though normal striate processing does not resemble normal parietal processing, much of the latter is dependent upon the former. However, there are at least two areas in the inferior parietal lobule that are independent of striate cortex, and each of these two areas has different visual properties: one behaviorally sensitive, the other behaviorally insensitive. This implies that the study of the posterior parietal cortex must therefore begin with an analysis of the region's functional diversity. The existence of redundant visual pathways implies that patients with damage to their cerebral association systems will still have visual capability in their cerebral association cortices, and this will encourage the development of new strategies of rehabilitation of patients with brain damage.

Proposed Course: The posterior parietal cortex of monkeys with striate damage will be examined further to verify the existence and location of the two areas of spared function already found. The oculomotor capability of animals with striate lesions will be studied quantitatively using the magnetic search coil method. A clinical protocol to study visual and oculomotor function in hemianopic patients has been submitted.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Neural Mechanisms)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00104-01 LSR						
PERIOD COVERED October 1, 1979, to September 30, 1980								
TITLE OF PROJECT (80 characters or less) Cerebellar-Dependent Adaptive Control of Saccadic Eye Movements								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Lance M. Optican</td> <td style="width: 33%;">Ph.D. Staff Fellow</td> <td style="width: 33%;">LSR NEI</td> </tr> <tr> <td>Other: Fred A. Miles</td> <td>Ph.D. Visiting Scientist</td> <td>LNP NIMH</td> </tr> </table>			PI: Lance M. Optican	Ph.D. Staff Fellow	LSR NEI	Other: Fred A. Miles	Ph.D. Visiting Scientist	LNP NIMH
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COOPERATING UNITS (if any) Department of Neurology, Johns Hopkins University Laboratory of Neurophysiology, NIMH								
LAB/BRANCH Laboratory of Sensorimotor Research								
SECTION								
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205								
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5						
CHECK APPROPRIATE BDX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The aim of this project is to increase our understanding of the neural bases of <u>adaptive mechanisms</u> responsible for maintaining appropriate performance of the <u>oculomotor system</u> . Previous work had already described an adaptive control mechanism for rapid, or <u>saccadic</u> , eye movements. This adaptive mechanism has two components: one which maintains saccadic accuracy and one which suppresses postsaccadic ocular drift. Hence, working in concert, these two components assure that saccadic eye movements accurately acquire visual targets and that these movements end abruptly, allowing clear vision. Both of these adaptive components depend upon an intact <u>cerebellum</u> . The mechanism for maintaining saccadic accuracy appears to be localized in the area of the posterior cerebellar vermis and paravermis. In this project the component of the adaptive mechanisms responsible for suppression of postsaccadic ocular drift were studied in detail. It was shown that full-field visual slip was sufficient to elicit adaptation in normal monkeys. This adaptive response was then studied in two monkeys with complete bilateral ablations of the <u>flocculus of the cerebellum</u> . The results showed that the ability of the brain to respond to the retinal slip <u>following saccades</u> had been reduced over tenfold.								

Project Description:

Objectives: The brain has the remarkable ability to continue performing a wide variety of tasks despite disturbances due to aging, disease or trauma. The way the brain compensates for these disturbances is important in understanding recovery from illness. The mechanisms which are themselves responsible for the compensation are especially interesting since it is often possible to appreciate the functional goal of a compensatory mechanism even when the function of the compensated system itself remains a mystery. The basic objective in this research is to attain a quantitative understanding of the neural bases of such adaptive mechanisms. The first step toward understanding the fundamental neural components of an adaptive mechanism is to quantify its performance. One area which lends itself to the quantification of compensation is that of eye movement control. The study of eye movements and the neural substrate for their generation has progressed to the point where detailed, quantitative questions can be asked. One oculomotor control system, that responsible for the rapid, or saccadic, eye movements used to change visual fixation, can be characterized fairly simply. Saccadic eye movements have high velocities (and hence brief durations) and the movements end abruptly. These features depend on a phasic burst, or pulse, and a tonic level, or step, of innervation. The pulse generates a large, brief force increase in the muscles which drives the eye rapidly against the viscous drag of the orbit. When the pulse cuts off, a new force must be established by the step to balance the elastic restoring forces of the orbit. If the step of innervation is not exactly matched to the pulse, the eye will not stop after the rapid part of the eye movement is over but will drift slowly to a different final position. These postsaccadic ocular drifts are suppressed by an adaptive mechanism which can be abolished in monkeys by total cerebellectomy.

We had previously established that retinal slip alone was sufficient to elicit drift suppression and that monkeys with lesions of the vestibular cerebellum (flocculus and nodulus) had enduring postsaccadic drifts. The aim of this year's research was to determine the range of this adaptive response in normal monkeys and to study the effects of bilateral floccular ablations on that adaptation.

Methods Employed: Rhesus monkeys were seated, with head fixed, before a translucent screen (subtending $100^\circ \times 100^\circ$). A densely featured image was back-projected onto this screen with a servo-controlled mirror galvanometer in the light path. The animals' eye movements were monitored with the Robinson search coil method and fed into a computer. Immediately after each saccade, the computer caused the mirror galvanometer to drift the image of the scene horizontally with an exponential time course and an amplitude proportional to that of the horizontal component of the antecedent saccade. In some experiments the scene was made to drift in the same direction as the saccade, and in others, in the opposite direction. The pulse-step mismatch, defined as the amplitude of the postsaccadic ocular drift divided by the magnitude of the antecedent saccade, provided a quantitative measure of the animals' postsaccadic drift. The same

experimental paradigm was then used to study the adaptive capability of monkeys following cerebellar ablations. Two monkeys were used in which bilateral removal of the cerebellar flocculus had been performed using suction ablation under direct visualization.

Major Findings: The results in normal monkeys established the range of, and some of the stimuli for, the adaptive mechanism responsible for postsaccadic drift suppression. All visual stimuli consisted of purely horizontal image motions. Three classes of stimuli were presented: pure displacements with no retinal image slip, slips with no net image displacement, and slips which resulted in a net displacement. The image slips were exponential with a time constant of about 50 msec. From experiments using these three classes of stimuli, it was possible to determine that retinal slip alone is sufficient to elicit an adaptive response. The adaptive response consisted of a zero latency, exponential postsaccadic ocular drift which was in the same direction as the optically-imposed postsaccadic retinal image slip. Image displacement alone is neither necessary nor sufficient to elicit an adaptive response. In response to exponential retinal image slips equivalent to a pulse-step mismatch of +50 percent (slip in direction opposite to antecedent saccade), a normal monkey was able to change his pulse-step mismatch to 5.5 percent within eight hours and to 14.1 percent after two days. When the slip was in the same direction as the antecedent saccade (equivalent to a pulse-step mismatch of -50 percent), a normal animal was able to change the pulse-step mismatch to -10 percent within eight hours and to -70 percent after three days.

After bilateral ablations of the cerebellar floccular lobes, monkeys were no longer able to achieve the same range of adaptation. Optically-imposed retinal image slip still produced an adaptive response although it was reduced almost tenfold in amplitude. In response to an imposed slip equivalent to +50 percent, a flocculectomized monkey was only able to change the pulse-step mismatch from -2 percent to +2 percent. In response to an imposed slip equivalent to a pulse-step mismatch of -50 percent, the pulse-step mismatch of a flocculectomized monkey only changed from +1 percent to -5 percent. Hence, bilateral ablations of the floccular lobes did not completely abolish the adaptive change of the pulse-step mismatch but did reduce its magnitude substantially so that the resultant eye movements were no longer able to compensate for the postsaccadic retinal slip.

Significance to Biomedical Research and the Program of the Institute: This research quantitatively defines a new area of oculomotor plasticity: the adaptive suppression of postsaccadic ocular drift. This opens up new areas for studying motor learning at the cellular level in the primate central nervous system. The oculomotor deficits that follow lesions of the cerebellar flocculus in monkey demonstrate the importance of this region in clinical diagnosis of neuro-ophthalmological disorders. The detailed knowledge of the performance of the system makes it possible to suggest

further tests which might aid in the localization of the pathology in human patients with classical cerebellar signs.

Proposed Course: The main thrust of these studies will continue to be in elucidating the neural bases for oculomotor plasticity. Two more parameters of the postsaccadic drift suppression mechanism will be quantified: the time course of the adaptive response and the nonlinear dependence of the eye movement on abnormal innervations. Subsequently, single-unit studies will be performed to investigate the information processing carried out in the cerebellum and brainstem during the adaptive process.

NEI Research Program: Sensory and Motor Disorders of Vision--Sensory and Motor Disorders Related to Specific Disease Processes

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00103-01 LSR										
PERIOD COVERED October 1, 1979, to September 30, 1980												
TITLE OF PROJECT (80 characters or less) Visual Processing in Prestriate Cortex												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Barry J. Richmond</td> <td style="width: 15%;">M.D.</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 10%;">LN</td> <td style="width: 9%;">NIMH</td> </tr> <tr> <td>Other: Robert H. Wurtz</td> <td>Ph.D.</td> <td>Chief</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI: Barry J. Richmond	M.D.	Medical Officer	LN	NIMH	Other: Robert H. Wurtz	Ph.D.	Chief	LSR	NEI
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COOPERATING UNITS (if any) Laboratory of Neuropsychology, NIMH												
LAB/BRANCH Laboratory of Sensorimotor Research												
SECTION 												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205												
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:										
1.0	0.0	1.0										
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SUMMARY OF WORK (200 words or less - underline keywords) <p>Behavioral experiments on monkeys have shown that the <u>inferior temporal cortex</u> is important for <u>pattern recognition</u>. <u>Single neurons</u> in this area of cortex are also responsive to visual stimuli. Our studies attempt to determine what parameters or features of visual stimuli are important for modulating the activity of cells in the inferior temporal cortex. Our initial survey shows that many neurons respond well to narrow slits of light of a particular orientation and that the excitatory receptive fields are small rather than large. Many of the cells also respond to <u>square wave</u> or <u>sine wave gratings</u>.</p>												

Project Description:

Objectives: The major visual pathway into the primate brain first reaches cerebral cortex in an area of the occipital lobe, the striate cortex, which in turn projects to other areas of the occipital, parietal and temporal lobes. One of these areas, the inferior temporal cortex, has been implicated in one type of visual processing, that for form vision--as opposed to color vision or movement perception. Behavioral experiments have shown that monkeys with ablations of the inferior temporal cortex are unable to perform visual discrimination tasks involving form and pattern recognition. In paralyzed anesthetized monkeys, some inferior temporal cells have also been shown to be responsive to visual stimulation in large areas of the visual field but many cells are unresponsive to conventional stimuli (spots of light, slits of light, dark bars). We have begun to study this area by using awake behaving monkeys and by changing the type of stimulation used to include in addition to conventional stimuli a periodic stimulus, either square wave or sine wave gratings. The responses of the cells to the conventional stimuli can then be compared to responses which have been studied extensively elsewhere in the visual system.

Methods Employed: Monkeys are trained to fixate a small spot of light in order that receptive fields of single neurons may be studied. Conventional visual stimuli (bars, slits, and spots) are produced by projection onto a tangent screen. Grating patterns are produced on a large (21 inch) oscilloscope screen by a hardware device controlled by a microprocessor; the system was designed and manufactured at the NIH by the Resource Services Branch of NIMH. Parameters of the grating display such as spatial frequency, drift rate, position in the visual field, contrast, and area of the visual field stimulated can be varied. The commands to change parameters come from the same computer which controls the overall experiment.

Major Findings: Since receptive fields of inferior temporal cortex neurons studied in paralyzed anesthetized monkeys had been found to be large and to always include the fovea, we were surprised to note that the receptive fields of the cells have been small, frequently less than 10 degrees in diameter. Receptive fields of 36 of 51 cells in posterior inferior temporal cortex frequently did include the fovea, but others stopped before reaching the edge of the fovea. The stimulus with which we have obtained the best response has been a narrow slit of light, typically one-quarter to one-half degree wide and one degree long. Some of the cells respond more strongly as the slit becomes longer--up to the 8 degrees which can be achieved in our apparatus. The response of other cells increases as the slit is lengthened (about 2-4 degrees) and then decreases as further lengthening occurs. The cells also have very tight orientation specificity or "tuning". If the orientation varies by as little as 15 degrees from the optimal orientation, the response to the stimulus may disappear entirely.

Many of the cells which respond to slit stimuli also show a response to grating stimuli of a relatively low spatial frequency (3-4 cycles/degree).

So far we have not found that sine or square wave grating stimuli are more potent stimuli for inferior temporal neurons than are slits and bars.

Significance to Biomedical Research and the Program of the Institute:

An understanding of how pattern processing takes place would lead to better diagnosis of the deficits suffered by people with damage to cortical brain areas and possibly reveal ways of how to better treat patients with these deficits.

Proposed Course: Recording from single neurons will be continued in order to quantify the observations described above. We will relate the properties of the neurons in the inferior temporal cortex to the properties of visually responsive cells in other more intensively investigated visual areas.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00045-02 LSR								
PERIOD COVERED October 1, 1979, to September 30, 1980										
TITLE OF PROJECT (80 characters or less) Visual and Eye Movement Properties of Neurons in the Pulvinar Nucleus										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: David Lee Robinson</td> <td style="width: 33%;">Ph.D. Research Physiologist</td> <td style="width: 15%;">LSR</td> <td style="width: 15%;">NEI</td> </tr> <tr> <td>Other: William Keys</td> <td>Ph.D. Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI: David Lee Robinson	Ph.D. Research Physiologist	LSR	NEI	Other: William Keys	Ph.D. Staff Fellow	LSR	NEI
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Other: William Keys	Ph.D. Staff Fellow	LSR	NEI							
COOPERATING UNITS (if any) Behavioral Sciences Department, Armed Forces Radiobiology Research Institute										
LAB/BRANCH Laboratory of Sensorimotor Research										
SECTION										
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:								
3.0	2.0	1.0								
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SUMMARY OF WORK (200 words or less - underline keywords) Investigations are being conducted into the <u>visual</u> and <u>eye movement properties</u> of neurons in the <u>pulvinar nucleus</u> of awake <u>monkeys</u> . Three groups of visual cells are present. <u>Pan-directional neurons</u> respond to stimuli moving across the receptive field in any direction. <u>Directionally selective cells</u> discharge to stimuli moving through the receptive field in several, but not all, directions. Some of these cells are inhibited by stimulus movement in a direction opposite to the preferred. <u>Oriented cells</u> discharge to stimuli moving in one direction or occasionally in two opposite directions. These cells only respond to stationary stimuli which are slits of light at specific orientations. The location of the receptive field of a cell moves with the eye; it does not remain constant in visual space as does visual perception. A small population of neurons in the pulvinar receive signals about eye movements because they discharge or are suppressed with eye movements made in the dark. Thus, there are pulvinar cells with visual responses, eye movement responses, or visual responses modulated by eye movements.										

Project Description:

Objectives: There are two major routes for visual information to reach the cerebral cortex of the primate brain. The first is from the retina to the lateral geniculate nucleus of the thalamus and then to the striate cortex which has been intensively studied. The second pathway is from the retina to the superior colliculus in the brainstem and then to the pulvinar nucleus of the thalamus. The pulvinar nucleus then projects to several different regions of the cerebral cortex. Although the visual properties of the superior colliculus have been studied extensively, the pulvinar remains largely unexplored. The cells in the superior colliculus which presumably project to the pulvinar are visually responsive and are strongly influenced by eye movements. This project is designed to study the processing of visual information beyond striate cortex and the superior colliculus. The work analyzes the types of visual stimuli which are most effective in influencing pulvinar cells and investigates what types of behavior modulate these visual responses.

Methods Employed: The discharge patterns of individual neurons in the pulvinar nucleus were recorded from awake, trained rhesus monkeys. The animals learned to fixate a spot of light projected onto a tangent screen in order to obtain water reinforcement. While the monkey fixated the spot of light, other lights were used to determine the visual properties of the cells. The size, shape, orientation, and movement of these visual stimuli were varied to determine what stimulus features caused the greatest change in a cell's discharge pattern. The animal also learned to make visually-guided eye movements from one light to another, and this allowed for analysis of oculomotor relationships of cells. Control of the animal's behavior was accomplished with a digital computer and data were analyzed on-line with the same instrument. After many recordings from an animal, the monkey's brain was perfused and sectioned to locate electrode recording sites. This procedure allowed for a correlation of physiological properties with anatomical location.

Major Findings: The vast majority of neurons in the pulvinar nucleus have a localized visual receptive field, a specific region of visual space within which light will influence the pattern of discharge of the cell. Neurons can be placed into three groups. First, oriented cells respond to stimuli moving in only one direction or in two opposite directions. They are selective for moving edges of light. Some of these neurons respond to stationary stimuli but only when they are slits of light or edges of specific orientations. These cells are capable of encoding the location and some of the physical characteristics of the stimuli which excite them. Second, pan-directional cells respond to stimuli moving in any direction and are not selective for the configuration of such stimuli. Many of these cells discharge to stationary stimuli of any size or shape. Such neurons appear to encode only the location of a stimulus and not its configuration. Third, directionally selective cells respond to the movement of any stimulus over several, but not all, directions. They are inhibited or give no

response to stimulus movement in nonpreferred directions. The discharge of these cells indicates the general direction in which a stimulus is moving.

The location of the visual receptive field of a pulvinar cell moves with the eye in a fixed relation to the fovea; for visual perception the location of an object in space remains stationary during eye movements. These data indicate that additional processing is required beyond the pulvinar and striate cortex for the mechanisms of perceptual constancy.

Many neurons in the pulvinar nucleus are excited or suppressed during or after visually-guided eye movements. For some of these cells, the eye movement associated activity disappears when the same eye movement is made in a darkened environment. For the remainder of the cells, the activity persists when visual factors are reduced. The latter cells appear to receive information about the occurrence of an eye movement suggesting that the pulvinar nucleus is in communication with some part of the oculomotor, as well as the visual, system.

Significance to Biomedical Research and the Program of the Institute: Vision is an active process. Visual stimuli are used to initiate and guide movement, and eye movement modifies vision. Different aspects of visual behavior may be mediated by different parts of the central nervous system. The experiments outlined here attempt to determine if specific types of visual behavior are localized within the pulvinar nucleus. Since this part of the thalamus has cells involved in processing visual information and neurons related to eye movements, the pulvinar nucleus appears to integrate the shifts in vision which are associated with each eye movement. Understanding the brain mechanisms of visual behavior and their neural correlates will facilitate the diagnosis of visual-motor and perceptual deficits in man.

Proposed Course: Future studies will attempt to correlate the visual properties of pulvinar cells with the behavioral and eye movement modulations demonstrated in previous work. The pulvinar is divided into four subdivisions and each has unique afferent and efferent connections. Additional experiments will be directed toward determining the functional organization of each of these subdivisions. These studies should help to identify the role of the pulvinar nucleus in the processes of active vision.

NEI Research Program: Sensory and Motor Disorders of Vision--Strabismus and Other Oculomotor Disorders (Disorders Affecting Control of Eye Movements)

Publications:

Robinson DL, Bushnell MC, Goldberg ME: The role of posterior parietal cortex in selective visual attention, in Fuchs AF, Becker W (eds): Proceedings of the Symposium on the Neural Control of Eye Movements. New York, Elsevier North-Holland (in press).

Goldberg ME, Robinson DL: The significance of enhanced visual responses in posterior parietal cortex. Behavior Brain Sci (in press).

Robinson DL, Keys W: Visuo-motor properties of neurons in superior colliculus and pulvinar nucleus of the monkey. Proc Int Union Physiol Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00043-02 LSR								
PERIOD COVERED October 1, 1979, to September 30, 1980										
TITLE OF PROJECT (80 characters or less) Projections of Area 17 to Inferior and Lateral Pulvinar in the Rhesus Monkey										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Leslie G. Ungerleider</td> <td style="width: 33%;">Ph.D. Senior Staff Fellow</td> <td style="width: 15%;">LSR</td> <td style="width: 15%;">NEI</td> </tr> <tr> <td>Other: Mortimer Mishkin</td> <td>Ph.D. Research Psychologist</td> <td>LN</td> <td>NIMH</td> </tr> </table>			PI: Leslie G. Ungerleider	Ph.D. Senior Staff Fellow	LSR	NEI	Other: Mortimer Mishkin	Ph.D. Research Psychologist	LN	NIMH
PI: Leslie G. Ungerleider	Ph.D. Senior Staff Fellow	LSR	NEI							
Other: Mortimer Mishkin	Ph.D. Research Psychologist	LN	NIMH							
COOPERATING UNITS (if any) Laboratory of Neuropsychology, NIMH										
LAB/BRANCH Laboratory of Sensorimotor Research										
SECTION										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205										
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The <u>sensory processing and perception</u> of visual information requires the transmission of neural activity across a multisynaptic pathway from <u>striate cortex</u> , or <u>area 17</u> , through several <u>prestriate</u> "association areas". In addition, visual input from <u>striate cortex</u> reaches these <u>prestriate</u> areas via two subcortical visual structures, the <u>superior colliculus</u> and the <u>pulvinar</u> . We have begun to explore the complex circuitry and <u>visuotopic</u> organization of these connections in the primate visual system by the combined use of <u>anterograde degeneration</u> and <u>autoradiographic tracing techniques</u> . Future experiments will examine efferents of <u>prestriate projection areas</u> to determine the pathways by which visual information is relayed from the <u>occipital lobe</u> to visual areas located within the <u>parietal and temporal lobes</u> .										

Project Description:

Objectives: Although the projections of lateral striate cortex, the part representing central vision, have been extensively studied in the rhesus monkey, little information exists regarding the projections of posterior and medial striate cortex, parts representing peripheral and far peripheral vision, respectively. We have previously described the cortical efferents from all parts of area 17, i.e., the locus, extent, and topographic organization of the entire striate-prestriate projection system. In the present investigation, we examined subcortical efferents from area 17 to the pulvinar, a nucleus in the thalamus implicated in attentional mechanisms.

Methods Employed: Anatomical material from two series of monkeys (*Macaca mulatta*) was used to determine the full extent and visuotopic organization of striate projections to the pulvinar. One series had been processed for terminal degeneration by the Fink-Heimer procedure following unilateral lesions of lateral, posterior, or medial striate cortex. Collectively, the lesions included all of area 17 with little or no invasion of area 18. The second series had been processed for autoradiography following tritiated amino-acid injections into striate sites representing the center of gaze and eccentricities ranging from 0.5° to 45° in either the upper or lower hemifield.

Major Findings: The results indicate a visuotopic organization of striate projections to the inferior and lateral pulvinar. The details of this organization are as follows. Within the rostral third of the inferior pulvinar (PI), striate projections representing a progression from central to far peripheral vision terminate in a progression from dorsolateral to ventromedial PI, with central vision represented at the dorsolateral margin of the nucleus, adjacent to the caudal tip of the dorsal lateral geniculate nucleus. The vertical meridian is represented at the lateral and dorsal borders of PI, while the horizontal meridian runs obliquely across the nucleus in a dorsolateral to ventromedial direction, with the lower hemifield represented dorsomedially and the upper hemifield, ventrolaterally. Missing from the rostral third of PI, however, are striate projections representing the fovea itself. These latter projections add a rostral-caudal dimension to the topography. Thus, whereas eccentricities greater than 5° are represented entirely within the rostral third of PI, those of 5° to about 2° extend into the middle third as well, and those of less than 2° occupy the middle and caudal third, with the center of gaze located at the caudal pole of the nucleus.

At the level of the caudal two-thirds of PI, where striate projections representing eccentricities of 5° or less terminate, the lateral pulvinar (PL) also receives a striate projection. This second projection originates only in the parts of striate cortex representing central vision, and appears to be a mirror image of the projection to adjacent PI, with the representation of the vertical meridian forming the common border. Whereas the striate projection zone occupies the entire rostral half of PI, it

occupies only a dorsal segment in the caudal half of this nucleus, and only a limited, adjacent segment of PL.

These present anatomical experiments show a precise retinotopic organization of striate cortex projections to PI and PL. Previous studies have shown visuotopic arrangement of striate and pulvinar projections to the prestriate cortical area OB. Together these studies indicate the existence of two sources of striate input to area OB that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via PI and PL.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the basic mechanisms mediating normal vision is the first step in the prevention, diagnosis, and alleviation of sensory and perceptual disorders. In particular, a delineation of the neural circuitry involved in the transmission of visual information beyond the striate cortex may promote advances in the treatment of residual visual function following injury, by either disease or acute assault, to the central visual pathways.

Proposed Course: The topographic organization of striate projections to both cortical and subcortical structures will be further examined in continued autoradiographic studies. By recording the activity of multiple units from the injection needle, tritiated amino acids will be placed into areas of striate cortex representing known parts of the visual field. This recording procedure will also be employed for investigating the corticocortical pathways from prestriate "association areas" into both the parietal and temporal lobes. In addition to the techniques of degeneration and autoradiography for anterograde tracing of neural connections, future experiments will employ horseradish peroxidase for tracing retrograde axonal transport. This latter technique will be particularly useful in determining the sources of input to the superior colliculus and the pulvinar. It is anticipated that the combined application of these procedures will help to unravel the apparent complexity in the pattern of projections within the primate visual system.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Neural Mechanisms)

Publications:

Ungerleider LG, Mishkin M: Two cortical visual systems, in Ingle DJ, Mansfield RJW, Goodale MA (eds): Advances in the Analysis of Visual Behavior. Cambridge, MIT Press (in press).

Ungerleider LG, Christensen CA: Pulvinar lesions in monkeys produce abnormal scanning of a complex visual array. Neuropsychologia 17:493-501, 1979.

Ungerleider LG, Mishkin M: The striate projection zone in the superior temporal sulcus of Macaca mulatta: Location and topographic organization. J Comp Neurol 188:347-366, 1979.

Laboratory of Vision Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1979 - September 30, 1980

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH
Jin H. Kinoshita, Ph.D.

One of the main objectives of conducting research in the Laboratory of Vision Research (LVR) is to uncover findings which may lead to an understanding of a disease process and eventually to its treatment. This report highlights the progress of three investigations which fulfill the principal objective of the LVR.

For almost two decades it has been known that there is present in retina a soluble substance (S-antigen) which when injected in experimental animals elicits a severe inflammatory reaction in the eye. This allergic type of reaction has been studied extensively because many of its features resemble those found in the human uveitis, a very serious and difficult clinical problem.

The S-antigen is tissue-specific and found in the photoreceptor layer of the retina. Investigators from different laboratories have been successful in purifying this antigen and in characterizing its properties. While purifying the S-antigen Dr. Shichi of the LVR was struck by the similarities in the properties of the S-antigen to rhodopsin kinase, an enzyme which he has been studying for a number of years. Both proteins were found in the retina loosely attached to the rod outer segment membranes. He found that the molecular weights of the two proteins were identical. Moreover, the purified rhodopsin kinase and S-antigen had similar immunological properties. S-antigen was shown to have kinase activity in that it catalyzed the phosphorylation of rhodopsin. Finally, the crucial experiment of demonstrating that rhodopsin kinase can induce uveitis similar to that by S-antigen in experimental animals was accomplished by NEI scientists, Drs. Gery and Nussenblatt. From these results it does appear that S-antigen and rhodopsin kinase are identical. The identification of S-antigen represents a major advance in ocular immunology.

The second notable study possibly leading to an understanding and treatment of a disease process is the investigation of a hereditary blinding disease in dogs that may provide a clue to the underlying cause of human retinitis pigmentosa. For many years Dr. Chader of the LVR has been examining the role of cyclic nucleotides in vision. Although much emphasis was first placed on cyclic AMP his studies revealed that cyclic GMP may play a more prominent role in the retina. When Irish setter dogs with inherited retinal degeneration were made available for study, Dr. Chader and associates focused

their attention on the enzymes involved in cyclic GMP metabolism. They found that the phosphodiesterase (PDE) enzyme which metabolizes cyclic GMP was deficient in the affected animals. The lack of this enzyme results in the accumulation of cyclic GMP and could account for the destruction of the photoreceptor cells. Thus, the intriguing possibility was that the long sought after gene defect in hereditary degeneration of the retina may be PDE. Further investigations, however, revealed that the situation is more complicated. The activity of PDE is activated by the protein called calmodulin. At birth, the form of retinal PDE is dependent on calmodulin. Normally with aging the PDE becomes independent of calmodulin for its activity. This conversion of a PDE requiring calmodulin to a form not dependent on the activator is important because with age calmodulin decreases. In the Irish Setter with retinal degeneration the transformation of PDE from one form to the other does not occur. Consequently, as calmodulin levels decline, there is too little PDE activity to prevent the accumulation of cyclic GMP which leads to destruction of cells. The possibility of correcting the defect by injecting calmodulin in the eye is currently being studied.

For this pioneering work, Drs. Chader and Liu of the LVR, Dr. Krishna of NHLB and Dr. Aguirre of the University of Pennsylvania School of Medicine received an award from The Fight for Sight organization.

The third laboratory study related to a clinical problem deals with diabetic cataracts. A series of experiments by LVR cataract researchers have established that the initiating factor in cataracts associated with diabetes and galactosemia is aldose reductase (A.R.). This enzyme activated when sugar levels are elevated converts sugars to polyols. Because polyols do not freely diffuse out of cells or are actively metabolized they accumulate to high levels causing cells to swell and eventually rupture. The osmotic changes caused by polyol retention initiates a series of events leading to opacification.

The most convincing evidence for the polyol-osmotic hypothesis came from in vivo experiments using inhibitors of aldose reductase. In galactosemic rats, systemic administration of an aldose reductase inhibitor effectively delayed the onset of cataract formation. Validation of the hypothesis from experiments with the diabetic animals had not been accomplished because diabetic rats require two or three months for cataracts to develop while in galactosemic rats only two weeks are required for cataracts. It was inconvenient to treat rats for the many months required for the formation of diabetic cataracts with only marginally active A.R. inhibitors. However, in the past year more potent A.R. inhibitors have been developed by pharmaceutical industries and with these inhibitors LVR scientists were successful in altering the course of diabetic cataracts in rats.

With Pfizer Company's Sorbinil it has been possible, for the first time, to delay effectively the development of cataracts in diabetic rats. The lens changes in the form of equatorial vacuoles appear within 3 weeks of diabetes, while the dense nuclear opacity requires 6-10 weeks. Diabetic rats treated with Sorbinil do not develop any lens changes, not even vacuoles, during 6 months of observation.

The course of cataract formation in galactose-fed rats is also strikingly blocked by Sorbinil treatment. Lenses of galactosemic rats develop vacuoles in 3 days and dense nuclear opacity within 2 weeks. However, with Sorbinil treatment, no lens changes occur during a 4 month study.

These results strongly suggest that with potent enough A.R. inhibitors it is possible not only to delay but also prevent the formation of sugar cataracts. The laboratory work with A.R. inhibitors forms the basis for the next stage of investigation - the testing of the A.R. inhibitors to alter the course of cataract formation in diabetic patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRANURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00003-08 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Cataracts		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Jin H. Kinoshita Other: Manuel Datiles Henry N. Fukui Peter Kador Howard Jernigan Lorenzo Merola	Ph.D. M.D. Ph.D. Ph.D. Ph.D.	Chief Visiting Scientist Research Chemist Research Chemist Research Associate Chemist
		LVR NEI LVR NEI LVR NEI LVR NEI LVR NEI LVR NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Biochemistry		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5.3	PROFESSIONAL: 4.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Current investigations are being conducted on the events leading to the formation of several types of cataracts. <u>Hereditary cataract</u> formation is being studied in two strains of mice developed in our laboratory. Both develop <u>osmotic cataracts</u> ; however, subtle significant differences suggest different modes of cataract formation in these strains. <u>Diabetic or sugar cataract</u> formation is also being studied. Initiated by the enzyme <u>aldose reductase</u> , methods for controlling the onset of these cataracts through the regulation of this enzyme are being developed.		

Project Description:

Objectives: To study the mechanism of cataract formation in laboratory animals and to develop means of regulating this cataractogenesis.

Methods Employed: The Nakano and Philly mouse strains with hereditary cataracts have been developed in our laboratories. Sugar cataracts can be induced in laboratory animals by making them diabetic with appropriate chemical agents, or by making them galactosemic or xylosemic with a diet enriched with either galactose or xylose.

Major Findings: In the study of congenital and hereditary cataracts the Philly mouse cataract is being studied in detail. Derived from the Swiss-Webster strain this mouse, developed in our laboratory, forms hereditary cataracts visible to the naked eye 5-6 weeks after birth. Although clear to the naked eye, slit lamp examinations reveal faint anterior opacities by the 15th postnatal day which progress to the suture area by the 25th day. By 30 days a nuclear opacity develops which surrounds the nucleus by day 35. At the same time an anterior subcapsular opacity becomes diffuse and pronounced as the cataract becomes obvious to the naked eye.

Biochemical studies indicate that an osmotic cataract is formed. By the 20th day there is an increase in lens water along with an alteration in the electrolyte levels. Concomitant with these electrolyte changes differential effects on the synthesis and degradation of crystallin proteins are seen. The total amounts of beta- and gamma-crystallins are reduced as measured by sodium dodecylsulfate polyacrylamide gel electrophoresis and radioimmunoassay. Laurell immunoelectrophoresis also indicates that the composition of beta-crystallin in Philly mice significantly differs from that of the normal mice. Protein synthesis as measured by the incorporation of [S-35] methionine also indicates a decrease in the incorporation of radio-label into beta and gamma - crystallins while incorporation into alpha-crystallin and non-crystallin proteins is not reduced.

Transport studies with alpha-aminoisobutyric acid (AIB) indicate no significant differences between Philly and control lenses. When rubidium is substituted for potassium a decreased accumulation of rubidium in Philly lenses older than 20 days can be correlated with increased rubidium leak-out. This decreased accumulation due to increased leak-out appears to be the key biochemical change that accounts for osmotic cataract formation and it suggests the possibility of a defect in membrane permeability.

In the Nakano mouse, another cataract animal model, a hereditary cataract is formed due to the presence of a polypeptide Na, K-ATPase inhibitor. The same polypeptide inhibitor can be obtained from Nakano lens epithelial cells grown in tissue culture. Through [S-35] methionine incorporation into tissue cultured lens epithelial cells the synthesis of this polypeptide ATPase inhibitor has been verified.

Studies on diabetic and galactosemic sugar cataracts are being continued. Initiated by the enzyme aldose reductase (alditol:NADP oxidoreductase, EC 1.1.1.21, AR) the onset of cataract formation can be delayed or controlled by the regulation of this enzyme. AR has also been postulated to be involved in initiating other diabetic complications such as neuropathy, nephropathy or retinopathy. Because of its role in initiating cataract and potentially other diabetic complications, intense research is being conducted by many pharmaceutical houses in the development of new AR inhibitors.

Aldose reductase from human placenta has been isolated by a series of chromatographic affinity columns and antibodies to the homogeneous enzyme have been raised in rabbits. The susceptibility of human placental aldose reductase (HPAR), human lens aldose reductase (HLAR) and rat lens aldose reductase (RLAR) to be inhibited has been compared through the use of 10 inhibitors of diverse structure. Previous studies comparing HPAR and RLAR had indicated significant differences in the susceptibility of AR to be inhibited and suggested that inhibitors designed for clinical use must eventually be evaluated with human enzyme. Current results indicate that all three enzymes differ in their susceptibility to inhibition suggesting that target tissue specificity must be taken into account in the design of AR inhibitors. Currently no 'universally potent' AR inhibitor exists.

In the development of enzyme inhibitors many classes of antiallergy drugs have been found to be AR inhibitors. Some of these inhibitors are structurally derived from the chromone inhibitors previously developed in our laboratory. Evaluation of the sterically nonconstrained R and S isomers of 1,3-dioxo-1H-benz [de]isoquinoline-2(3H)-2' - propionic acid and the sterically constrained enantiomers of 6-fluoro-spiro[chroman-4,4'-imidazolidine]-2', 5'-dione indicated that aldose reductase stereochemically recognizes the inhibitors at an inhibitor site independent of the substrate and NADPH cofactor site.

Studies on the transport of organic compounds into the lens have also been initiated. Through these studies a choline transport system in the lens has been discovered. This system through apparent active transport can concentrate choline, ethanolamine and other analogs to lenticular levels substantially higher than in the surrounding lens medium. Potential photooxidative damage to cultured lenses produced by the presence of photosensitizing organic molecules was also studied. Results indicate that the carrier mediated transport systems could be affected by the presence of either hydrogen peroxide, produced in the presence of the photosensitizer riboflavin or singlet oxygen produced in the presence of rose bengal. Of the transport systems evaluated the choline system was found to be more sensitive to photooxidative damage than either the AIB or cation transport systems.

The in vivo ability of aldose reductase inhibitors to delay sugar cataract formation has been evaluated in both the galactosemic cataract model and the diabetic rat model. In the galactose cataract model rats fed a diet of either 30% or 50% galactose develop dense nuclear cataracts within

2-3 weeks. In contrast rats made diabetic with streptozotocin develop dense nuclear cataracts at a longer and more variable period of 6-9 weeks. Using the potent inhibitor Sorbinil (6-fluoro-spiro [chroman-4,4'-imidazolidine]-2',5'-dione) no lenticular changes could be detected for a period of up to 6 months when 60 mg/kg/day of the drug was administered by intubation to diabetic rats. This indicates that AR inhibitors of appropriate potency can significantly delay diabetic cataract formation.

Significance to Biomedical Research and the Program of the Institute:

Although cataract can be corrected with surgery, vision loss due to cataract presents a major public health problem. Worldwide, cataract is one of the major causes of blindness. By understanding the biochemical changes associated with cataract development in animal models methods for the delay or prevention of cataract may be uncovered. Moreover, through the study of sugar cataracts and aldose reductase regulation methods for the control of diabetic cataract formation and possibly the control of other diabetic complications can be developed.

Proposed Course: These projects will be continued. In the Philly mouse membrane composition and permeability characteristics will be further explored. In the Nakano mouse the polypeptide Na K-ATPase inhibitor will be characterized.

The mechanism of action of aldose reductase inhibitors will be studied in detail along with the structure-activity relationships of the inhibitors. Through such studies the minimum requirements of the inhibitory site may be determined so that more active inhibitors may be designed.

NEI Research Program: Cataract--Diabetic Cataract/Congenital, Metabolic, and Genetic Cataracts

Publications:

Fukui HN, Merola LO, Fukushima S, Kinoshita JH: Nakano mouse cataract. Red blood cell and lens metabolism symposium. Edited by Satish K. Srivastava. Elvsevier North Holland, Inc. New York 1980 (in press).

Fukushi S, Merola LO, Kinoshita JH: Altering the course of cataracts in diabetic rats. Invest Ophthalmol Vis Sci 19:313-315, 1980.

Fukushi S, Merola LO, Tanaka M, Datiles M, Kinoshita JH: Reepithelization of denuded corneas in diabetic rats. Exp Eye Res (in press).

Kador PF, Jernigan HM, Kinoshita JH: Accumulation and incorporation of radiolabeled choline into cultured rabbit lenses: Evidence for a choline transport system. Exp Eye Res 30:1-11, 1980.

Kador PF, Fukui HN, Fukushima S, Jernigan HM, Kinoshita JH: Philly mouse: A new model of hereditary cataract. Exp Eye Res 30:59-68, 1980.

Uga S, Kador PF, Kuwabara T: Cytological study of the Philly mouse cataract. Exp Eye Res 30:79-82, 1980.

Piatigorsky J, Kador PF, Kinoshita JH: Differential synthesis and degradation in the hereditary Philly mouse cataract. Exp Eye Res 30: 69-78, 1980.

Kador P, Uga S, Piatigorsky J: The Philly mouse hereditary cataract. Aging of the lens. In Regnault F, Hockwin O, Courtois Y (eds); Elvsevier/North Holland Biomedical Press, New York, 1980, pp 157-170.

Kador PF, Kinoshita JH, Tung WM, Chylack LT: Differences in the susceptibility of various aldose reductases to inhibition (II). Invest Ophthalmol Vis Sci (in press).

Chiou SH, Chylack LT Jr, Bunn HF, Kinoshita JH: Role of non-enzymatic glycosylation in experimental cataract formation. Biochem Biophys Res Commun (in press).

Kinoshita JH, Fukushima S, Merola LO, Datiles MB: Blocking sugar cataract formation with sorbinil. Invest Ophthalmol Vis Sci 19 (ARVO Suppl):12, 1980.

Fukui HN, Jernigan HM, Goosey JD, Kinoshita JH: Peroxidative effects on the choline uptake by the lens. Invest Ophthalmol Vis Sci 19(ARVO Suppl):13, 1980.

Kador PF, Goosey JD, Kinoshita JH, Sharpless NE: Antiallergy drugs as aldose reductase inhibitors. Invest Ophthalmol Vis Sci 19(ARVO Suppl):12, 1980.

Datiles MB, Fukui HN, Kinoshita JH, Fukushima S, Carter-Dawson L: Corneal reepithelization in the galactosemic rat. Invest Ophthalmol Vis Sci 19(ARVO Suppl):75, 1980.

Jernigan HM, Kador PF, Kinoshita JH: Transport and phosphorylation of choline in rat lens. Invest Ophthalmol Vis Sci 19(ARVO Suppl): 266, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00035-02 LVR	
PERIOD COVERED October 1, 1979, to September 30, 1980			
TITLE OF PROJECT (80 characters or less) Effects of Rod Outer Segments on Cells in Culture			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Igal Gery	Visiting Scientist	LVR NEI
Other:	Gerald Chader Elena Barraquer Paul O'Brien Julia Derr	Research Chemist Guest Worker Research Chemist Biologist	LVR NEI LVR NEI LVR NEI LVR NEI
COOPERATING UNITS (if any) None			
LAB/BRANCH Laboratory of Vision Research			
SECTION Sections on Biochemistry and Retinal & Corneal Metabolism			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205			
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Retinal rod outer segments</u> (ROS) contain high levels of compounds which may become toxic upon <u>oxidation</u> and <u>damage</u> the retina and other ocular tissues; this process is assumed to take place in diseases like retrolental fibroplasia. The mechanisms which normally prevent this oxidation are not known, and the possible role of <u>pigment epithelium</u> (PE) cells was tested here. PE cells were found to be resistant to ROS in culture at concentrations which were markedly toxic to cells like lymphocytes. Furthermore, PE cells provided a partial protection to lymphocyte cultures affected by ROS. </p> <p> The <u>retinopathy</u> of RCS rats is attributed to deficiency of their PE cells to <u>phagocytose</u> ROS. The possibility that <u>macrophages</u> (Mφ) of these rats are also deficient in their capacity to phagocytose ROS was tested and ruled out: Mφ from RCS rats resembled Mφ from all control rats in their phagocytosis of ROS. </p>			

Project Description:

Objectives: We have previously shown (FY 1979) that preparations of rod outer segments (ROS) are highly inhibitory to cultures of certain cells, seemingly by releasing toxic oxidation products. Pigment epithelial (PE) cells normally phagocytose ROS tips and, therefore, the possibility that PE cells are equipped with potent antioxidant capacity was tested.

The hereditary retinal dystrophy of RCS rats is attributed to a deficiency in their PE cells to phagocytose ROS. The possibility that macrophages (M ϕ) of these rats are also deficient in their capacity to phagocytose ROS has been indicated in the literature (Essner and Gorrin, 1979) and was examined here.

Methods Employed: The effects on cell cultures of bovine ROS and the combination of xanthine and xanthine oxidase (a source for free radicals) were determined according to the reduction in thymidine incorporation by the affected cells. Tested cells included chicken embryo PE, mouse lens epithelium, mouse or guinea pig spleen lymphocytes, or P815 cell line. The protective capacity of cells resistant to ROS was tested by adding these cells to lymphocyte cultures inhibited by ROS.

Peritoneal M ϕ from RCS and other rat strains were collected either without any treatment of donors, or following induction with proteose peptone. ROS labeled with isotopes in vivo (rat) or in vitro (bovine) were added to M ϕ monolayers and their uptake was measured according to the level of radioactivity retained after washing.

Major Findings: PE cells were resistant to ROS at concentrations which were highly inhibitory to lymphocytes or P815 cells. Addition of PE cells to lymphocyte cultures provided some protection against the inhibitory effects of ROS. Free radicals generated by xanthine and xanthine oxidase were highly inhibitory to all cell cultures, with small differences being noted between the various tested cells.

Peritoneal M ϕ from RCS rats resembled M ϕ from healthy RCS controls or from other normal rat strains in their capacity to phagocytose labeled ROS. This similarity in phagocytic activity was observed with ROS from either rat or bovine retinas.

Significance to Biomedical Research and the Program of the Institute. The capacity of ROS to generate toxic free radicals is assumed to play a pathogenic role in diseases like retrolental fibroplasia. It seems important, therefore, to learn about the mechanisms by which the oxidative processes are triggered and prevented in vivo. Our data are in line with the hypothesis that PE cells are resistant to the cytotoxic effects of ROS and may even provide antioxidative activity to surrounding tissues. It is noteworthy, however, that the antioxidative activity of cultured PE cells is inferior to that of compounds like vitamin E, catalase, or superoxide dismutase (data on these antioxidants

are described in our FY 1979 report). The latter agents are present in high concentrations in the retina and may thus play the major role in preventing damaging oxidative processes.

Our data with M ϕ from RCS rats provide evidence to rule out the suggested notion that the genetic lesion in RCS rats affects the phagocytosis of ROS by all phagocytic cells of these animals. Thus, the lesion seems confined to PE cells only.

Proposed Course: The phagocytosis of ROS and other particles by PE cells will be studied in vitro, in particular with regard to the similarities between PE cells and M ϕ . Agents known to modulate phagocytosis by M ϕ will be tested on PE cells; these studies should provide information concerning the processes involved in the phagocytosis by PE cells, as well as the possibilities of regulating these processes by exogenous agents.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium.

Publications:

Gery I: Inhibition of DNA and RNA synthesis in lymphocyte cultures by rod outer segments and its counteraction by vitamin E and other anti-oxidants. Invest Ophthalmol Vis Sci 19:751-759, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00023-02 LVR												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Macrophage Interactions with Storage Lipids														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Igal Gery</td> <td style="width: 35%;">Visiting Scientist</td> <td style="width: 15%;">LVR NEI</td> </tr> <tr> <td>Other:</td> <td>John A. Barranger</td> <td>Chief, Clinical Section</td> <td>DMNB NINCDS</td> </tr> <tr> <td></td> <td>Julia Derr</td> <td>Biologist</td> <td>LVR NEI</td> </tr> </table>			PI:	Igal Gery	Visiting Scientist	LVR NEI	Other:	John A. Barranger	Chief, Clinical Section	DMNB NINCDS		Julia Derr	Biologist	LVR NEI
PI:	Igal Gery	Visiting Scientist	LVR NEI											
Other:	John A. Barranger	Chief, Clinical Section	DMNB NINCDS											
	Julia Derr	Biologist	LVR NEI											
COOPERATING UNITS (if any) Clinical Section, Developmental and Metabolic Neurology Branch, NINCDS														
LAB/BRANCH LVR														
SECTION Biochemistry														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205														
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.5	OTHER: 0.6												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <p> The effects of <u>lipids</u> which accumulate in <u>storage diseases</u> were studied at the cellular level. <u>Mouse macrophage (Mϕ)</u> cultures took up large amounts of <u>gluco-cerebroside (GL₁)</u>, a lipid which accumulates in <u>Gaucher's disease</u>. Consequently, Mϕ exposed to GL₁ released high levels of <u>lysosomal enzymes</u> and a mediator, <u>lymphocyte activating factor (LAF)</u>. The interaction between GL₁ and Mϕ was found to be highly selective: (a) other storage lipids, like <u>sphingomyelin</u> or <u>GM₂ ganglioside</u>, were taken up by Mϕ to a much smaller amount than GL₁ and had minimal or no effect on the release of Mϕ products; (b) unlike Mϕ, cultures of other cell types, lens epithelium or skin fibroblasts, failed to accumulate significant quantities of GL₁. This selectivity in interaction between GL₁ and Mϕ in culture is in line with the exclusive accumulation of GL₁ in Gaucher's patients, in Mϕ of the reticuloendothelial system. In other experiments it was found that <u>Mϕ activated in vivo by bacteria</u> or other agents lost considerably their capacity to accumulate GL₁ and to release products when incubated with this lipid. GL₁ may be useful, therefore, in differentiating between untreated and activated Mϕ. </p>														

Project Description:

Objectives: Storage diseases often affect the eye. This project has been aimed at studying the effects of storage lipids at the cellular level and in a previous report (FY 1979) we have recorded initial findings concerning the effects of glucocerebroside (GL₁), the storage material of Gaucher's disease, on macrophage (M ϕ) cultures. These studies have been extended, in particular with regard to the selectivity of the interaction between the tested lipid and the cell in culture. In addition, the relationship between the level of activation of M ϕ and their reactivity to GL₁ was tested.

Methods Employed: Monolayers of enriched mouse peritoneal M ϕ were obtained by routine methods. Uptake of isotope-labeled storage lipids or rod outer segments was measured in monolayers of M ϕ , mouse lens epithelium (a cell line), or human skin fibroblasts (subcultures). The effects of lipids or other agents on M ϕ were determined according to their capacity to increase the release of lysosomal enzymes (mainly hexosaminidase) and a mediator, lymphocyte activating factor (LAF), which increases the mitotic activity in mouse thymocytes. Activated M ϕ were obtained by injecting donor animals with BCG bacteria, thioglycollate, or sea star factor (provided by Dr. R. Prendergast, Wilmer Inst., Johns Hopkins Univ.).

Major Findings: M ϕ cultures take up large amounts of GL₁ and the accumulation of the lipid increased the release of lysosomal enzymes and the mediator LAF. The interaction between GL₁ and M ϕ was found to be highly selective since (a) other storage lipids, sphingomyelin (SPM), GM₂-ganglioside or ceramide trihexoside, had no detectable effect on the release of the M ϕ products; (b) M ϕ cultures failed to accumulate significant amounts of lipids other than GL₁ and (c) unlike M ϕ , lens epithelium or skin fibroblasts took up just minute amounts of GL₁, while being comparable to M ϕ in their capacity to accumulate particles like retinal rod outer segments.

M ϕ activated in vivo by bacteria or other agents were markedly inferior to untreated ("resident") M ϕ in their capacity to accumulate GL₁ and to increase consequently their release of enzymes or LAF.

Significance to Biomedical Research and the Program of the Institute: The aforementioned findings provide new information concerning the effects of storage lipids at the cellular level. Of particular interest is the accordance between the selective uptake of GL₁ in M ϕ in culture and the exclusive accumulation of this lipid in the phagocytes of the reticulo-endothelial system in Gaucher's disease. (Other tested lipids, like SPM or GM₂, accumulate in cells other than M ϕ in the corresponding diseases, Fabry's or Tay-Sachs). It is conceivable that research of the interactions between lipids and cells in culture may help understanding the pathogenesis of these diseases, many of which affect the vision system.

The finding concerning the difference between "resident" and "activated" M ϕ in their interaction with GL₁ provides a new parameter for defining the

changes which accompany the M ϕ activation. In addition to their role in defense mechanisms against infection or malignancy, activated M ϕ are a major component of inflammation (particularly of the chronic type) in ocular and other tissues. Knowledge about these M ϕ should be useful for studies concerning the inflammatory process.

Proposed Course: The interaction between M ϕ and GL₁ will be further studied in the following lines: (a) the mechanisms involved in the selective accumulation of this lipid, mainly with regard to the hypothetical existence of a specific receptor on the M ϕ and the possible role of sugars in this interaction; (b) the biochemical and ultrastructural changes which take place in the affected M ϕ . In parallel, the reactivity of human monocytes in culture to storage lipids will be tested and compared to that of the mouse M ϕ .

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders--Inflammatory Disorders.

Publications:

Ben-Zvi A, Rodrigues M, Schiffmann E, Gery I: Induction of inflammation by a synthetic mediator. Fed Proc 39:319, 1980.

Bonney RJ, Davies P, Staruch MJ, Gery I, Kuehl FA, Humes JL: Possible roles of prostaglandins synthesized and secreted by macrophages in regulating immune responses. Agents and Actions (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00136-08 LVR																		
PERIOD COVERED October 1, 1979, to September 30, 1980																				
TITLE OF PROJECT (80 characters or less) Chemistry and Metbolism of the Lens																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">Paul Russell</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 25%;">Research Chemist</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Jin H. Kinoshita</td> <td>Ph.D.</td> <td>Chief</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Ann Rosman</td> <td>B.A.</td> <td>Biologist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI	Other:	Jin H. Kinoshita	Ph.D.	Chief	LVR	NEI		Ann Rosman	B.A.	Biologist	LVR	NEI
PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI															
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COOPERATING UNITS (if any) None																				
LAB/BRANCH Laboratory of Vision Research																				
SECTION Section on Biochemistry																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																				
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.3	OTHER: 1.0																		
CHECK APPROPRIATE BOX(ES)																				
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																				
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SUMMARY OF WORK (200 words or less - underline keywords)																				
<p>Studies on proteins from <u>human and animal lenses</u> have continued. A new method for the isolation of the <u>intrinsic membrane proteins</u> in lens has been devised. With this method the changes in the membrane proteins can be followed during <u>cataract development</u> without excessive contaminating cytoplasmic proteins obscuring the results. Because the membrane polypeptides retain their <u>immuno-reactivity</u>, these membrane components can be more fully characterized.</p> <p><u>Culturing</u> of the <u>epithelial cells</u> of the lens was also performed. Studies with animal lens cells in tissue culture have focused on the isolation of an <u>inhibitor of the Na-K ATPase</u>. Studies on <u>human lens epithelia</u> have been undertaken to determine activities of different <u>enzyme</u> in the glycolysis pathway in vitro.</p>																				

Project Description:

Objectives: To investigate the processes involved in the formation of cataract, the study of the changes in lens components is necessary. One of the major changes observed with many cataracts is an alteration in membrane permeability. Changes in the membranes of lenses from animal sources as well as human specimens are studied in hopes that a correlation can be made between development of cataract and specific membrane protein changes. Cell culture is also attempted to investigate specific parameters of epithelial cells from the lens.

Methods Employed: Proteins from the lens as well as enzymatic activities from cells in the lens have been studied. Spectroscopic, gel electrophoretic and immunochemical methods have been employed. Electron microscopic methods in collaboration with the LVR Experimental Pathology Section have also been used.

Major Findings: The main intrinsic membrane protein from young animal or human lenses is a 26,000 MW polypeptide. This polypeptide is isolated from the gap junction enriched fraction. With lenses from older lenses a 23,000 MW component is also seen. One of the difficulties with the study of cataractous lenses is the large amount of closely associated crystallin components which are present in the membrane fraction. This crystallin material obscures the resolution of the membrane polypeptides. In addition, the isolation of these membrane constituents is difficult and time consuming.

To alleviate the problems encountered with membrane protein isolation, a new method has been devised which not only reduces the isolation procedure from four days to one day but also eliminates much of the cytoplasmic protein material. This alkali extraction allows analysis by sodium dodecyl sulfate gel electrophoresis of lens membrane proteins even from cataractous lenses.

The alteration or decrease of the main intrinsic membrane polypeptide (26,000 MW) in cataractous mouse lenses occurs concomitant with the decrease in particles within gap junctions between fiber cells. These gap junctions normally electrically couple one cell with another. The appearance of the 23,000 MW polypeptide correlates with the disappearance of the 26,000 MW one in cataractous rodent lenses. These results suggest a possible post translational modification of the 26,000 MW polypeptide to the 23,000 MW one.

The alteration in the membrane protein is occurring when other changes are occurring in the lens. In Nakano and Philly mice, two cataractous mouse strains, leakage of crystallin protein occurs at about the time when the 26,000 MW polypeptide is decreasing. Heavy molecular weight aggregates are forming and many lens crystallin polypeptides become closely associated with

the cell membrane at this time also. The rapid decrease in 26,000 MW component in both strains of cataractous mice as well as with cataractous rat lenses suggests that this alteration is one of the changes which may occur in the development of human cataract.

In addition to changes in membrane proteins, whole human lenses removed for cataract extraction or lenses from eye bank eyes were utilized to investigate changes in lens proteins during cataract development as well as to study properties of lens epithelial cells in culture. Cataractous human lenses have shown a decrease in the lower molecular weight polypeptides when compared to normal eye bank lenses using sodium dodecyl sulfate polyacrylamide gel electrophoresis. This decrease is similar to the decreases seen with mouse lenses during cataract development. In the mouse, the loss of the low molecular weight polypeptides has been shown to be a result of insolubilization of these proteins, degradation, and leakage from the lens. Aqueous humor samples taken from patients prior to cataract have thus far failed to show any immuno reactive crystallin material. The crystallin could be leaking from the lens, however, at levels lower than what can be detected by current immunochemical methods. Aggregation, insolubilization and degradation are possibilities which we are pursuing.

The conditions for culturing the epithelial cells of the lens have been investigated. Use of collagen coated growth surfaces and addition of known mitogens have had little effect on the growth of the lens cells in culture. Although some growth is possible, cells from human lenses generally undergo rapid senescence in culture. Altering of the growth conditions which have assisted other primary cells in adapting to the tissue culture environment are being attempted. Analysis of lens epithelial cells from a patient whose family displays hereditary congenital cataract revealed large deposits of glycogen and a decreased activity of some enzymes in the glycolysis pathway.

Significance to Biomedical Research and the Program of the Institute: Investigation of the alterations that occur during development of cataract is necessary to understand the mechanisms involved in opacification. The changes in the membranes, particularly in the gap junctions, may proceed or occur concomitant with other changes previously observed during cataractogenesis. The modification of intrinsic membrane polypeptides that occurs in the animal models during cataract is seen although to a lesser extent in the aging human lens. Understanding of the reason for this change may be important to our knowledge of human cataractogenesis.

Use of appropriate tissue culture conditions would allow investigation of specific defects in the lens with a larger sample size than is currently available. This technique allows isolation of defective proteins from human cataractous lenses that is currently not possible.

Proposed Course: Use of in vitro condition to stimulate the changes in membrane polypeptides will be attempted. Determination of the quantity of intrinsic membrane polypeptide with rocket immunoelectrophoresis will

also be undertaken. The relationship between the membrane polypeptide and the cell cytoskeleton is another important area for future research. Culture of epithelial cells will be attempted to explore the factors responsible for the rapid senescence of lens epithelial cells in vitro.

NEI Research Program: Cataract--The Normal Lens

Publications:

Russell P, Fukui HF, Kinoshita JH: A Na-K ATPase inhibitor from cultured lens cells. Proceeding of the second international symposium of the red blood cell and the lens (in press).

Russell P, Fukui HF, Kinoshita JH: Properties of a Na-K ATPase inhibitor in cultured lens epithelial cells. Proceeding of the ocular tissue culture symposium. Vis Res (in press).

Russell P, Uga S, Zigler JS Jr, Kaiser-Kupfer M, Kuwabara T: Studies using human lens from a family displaying hereditary congenital cataracts. Proceedings of the ocular tissue culture symposium. Vis Sci (in press).

Zigler JS Jr, Carper DA, Russell P, Kinoshita JH: Analysis of immuno-chemical properties of human β -crystallin by radioimmunoassay. Exp Eye Res (in press).

Russell P, Kinoshita JH: Changes in the mouse membrane during cataractogenesis. Invest Ophthalmol Vis Sci 19(ARVO Suppl): 150, 1980.

Carper DA, Zigler JS Jr, Russell P, Kinoshita JH: Application of Laurell immunoelectrophoresis to the quantitation of lens crystallins. Invest Ophthalmol Vis Sci 19(ARVO Suppl):207, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00007-05 LVR										
PERIOD COVERED October 1, 1979, to September 30, 1980												
TITLE OF PROJECT (80 characters or less) The Biochemical Pharmacology of the Eye												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">Hitoshi Shichi</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Research Chemist</td> <td style="width: 20%;">LVR NEI</td> </tr> <tr> <td>Other:</td> <td>Daniel W. Nebert</td> <td>M.D.</td> <td>Chief</td> <td>DPB NICHD</td> </tr> </table>			PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR NEI	Other:	Daniel W. Nebert	M.D.	Chief	DPB NICHD
PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR NEI								
Other:	Daniel W. Nebert	M.D.	Chief	DPB NICHD								
COOPERATING UNITS (if any) Developmental Pharmacology Branch, NICHD												
LAB/BRANCH Laboratory of Vision Research												
SECTION Section on Biochemistry												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205												
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0										
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) Drug metabolizing enzymes such as <u>aryl hydrocarbon hydroxylase</u> and <u>enzymes for mercapturate formation</u> (glutathione S-transferase, γ -glutamyl transpeptidase, cysteinyl glycine peptidase and N-acetyl transferase) are localized rather exclusively in the <u>ciliary body</u> and <u>pigmented epithelium</u> . The enzymes distribute in different microsomal subfractions.												

Project Description:

Objectives: Distribution of drug metabolizing enzyme activities in microsomal subfractions was studied.

Methods Employed: Fresh bovine eyes were dissected to collect various ocular tissues. Microsomal fractions were prepared and further fractionated into subfractions in a sucrose gradient. Drug metabolizing enzyme activities were then determined.

Major Findings: Activities of drug metabolizing enzymes such as aryl hydrocarbon hydroxylase, UDP-glucuronyl transferase, glutathione S-transferase, gamma-glutamyl transpeptidase, cystine aminopeptidase, N-acetyl transferase are high in the ciliary body and pigmented epithelium. Examination of the enzymes for mercapturate formation in microsomal subfractions indicates that γ -glutamyl transpeptidase and aminopeptidase are collected in the same fractions but N-acetyl transferase is not. Therefore, these enzymes, although they catalyze the reaction sequentially, are not present as a single enzyme complex in microsomes.

Significance to Biomedical Research and the Program of the Institute: Little is known about the fate of drugs and environmental chemicals entering the eye through blood circulation or by topical administration. Elucidation of the mechanism of drug metabolism in the eye provides important knowledge in designing drugs for ocular diseases and for prevention of drug toxicity in the eye.

Proposed Course: Roles of drug metabolizing enzymes for normal ocular function will be investigated.

NEI Research Program: Retinal and Choroidal Diseases--Special Areas of Future Interest (Toxic and Environmental Disorders).

Publications:

Das ND, Shichi H: Gamma-glutamyl transpeptidase of bovine ciliary body: Purification and properties. Exp Eye Res 29:109-121, 1979.

Das ND, Shichi H: Tissue differences in gamma-glutamyl transpeptidase attributed to sialic acid content. Life Sci 25:1821-1828, 1980.

Shichi H, Tanaka M, Nebert DW: Genetic differences in cataract and other ocular abnormalities induced by paracetamol and naphthalene. Pharmacology 20:229-241, 1980.

Shichi H, Nebert DW: Drug metabolism in ocular tissues: Extrahepatic Metabolism of Drugs and Other Foreign Compounds, Gram TE, (ed): Spectrum Publications, Inc. Jamaica, NY, pp 333-364.

Shichi H: Distribution and function of drug-metabolizing enzyme in the eye. The Proceedings of the 2nd Natl. Congress of Eye Res. Japanese Chapter of the ISER pp 2-4, 1980.

Das ND, Shichi H: The distribution and role of drug-metabolizing enzymes in the eye. Invest Ophthalmol Vis Sci 19(ARVO Suppl):186, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00004-06 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) The Biochemistry of the Visual Process		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Hitoshi Shichi • Charles N. Rafferty	Ph.D. Research Chemist LVR NEI Ph.D. Research Chemist LVR NEI
Other:	Hiroyuki Fukui Robert L. Somers	M.D. Postdoctoral Fellow LVR NEI B.S. Chemist LVR NEI
COOPERATING UNITS (if any) Institute for Protein Research, Osaka University, Osaka, Japan		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Biochemistry		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 1.5	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Rhodopsin kinase</u>, an enzyme that catalyzes <u>phosphorylation</u> of <u>rhodopsin</u> with <u>ATP</u> in the <u>light</u>, was identified with a <u>retina-specific soluble antigen (S-antigen)</u> which causes <u>autoimmune uveitis</u> in experimental animals. <u>Rhodopsin kinase</u>, <u>cyclic nucleotide phosphodiesterase</u> and <u>GTP binding protein</u> are localized on the <u>external surface</u> of the disk membrane. These enzymes, together with <u>rhodopsin</u>, form a functional complex. While no <u>GTPase</u> activity is shown by a mixture of purified <u>GTP binding protein</u>, <u>rhodopsin</u> and <u>GTP</u> in the dark, the activity becomes manifested in the light. The <u>N-terminus</u> of <u>rhodopsin</u> was identified as <u>N-acetylmethionine</u>. Evidence is obtained that the <u>chromophore</u> of <u>rhodopsin</u> is <u>protonated</u> by <u>hydronium ion</u> and <u>proton translocation</u> accompanies <u>metarhodopsin I</u> formation. </p>		

Project Description:

Objectives: The objectives of this project are to investigate the light-dark adaptation processes of the retina by means of modern techniques of biochemistry and membrane biology. More specifically, these are (1) identification of a sequence of molecular events initiated by absorption of photons and leading to visual transduction (light process) and (2) elucidation of the biochemical mechanism for regenerating the photosensitivity of photoreceptor membranes (dark process). The investigations presented in this report deal with two aspects of the photoreceptor membrane proteins, i.e. (a) the location and function of enzymes involved in nucleotide metabolism and (b) structural features of rhodopsin.

Methods Employed: Biochemical methods such as centrifugation, column chromatography, spectroscopic analysis, and radioisotope assay.

Major Findings: I. Location and function of enzymes involved in nucleotide metabolism. (a) Rhodopsin kinase. Bovine rhodopsin kinase was purified to homogeneity by ammonium sulfate fractionation and chromatography on Sephacryl S-200 and Blue Sepharose. The purified enzyme (Mol. Wt. = 50,000-53,000) was independent of cyclic nucleotides for its activity, was specific for rhodopsin, and preferred ATP to GTP as the phosphate donor. Light was required to form the substrate opsin from rhodopsin. About 5 phosphates were incorporated per mol rhodopsin. Only ca. 20% of the total rhodopsin in the rod was phosphorylated. This was related to the finding that the phosphorylation reaction was primarily associated with newer disks rather than older disks. This is the first evidence that disks are biochemically heterogeneous. Using intact and inverted disks, rhodopsin kinase was localized on the external surface of the disk.

Experimental autoimmune uveitis is known to be caused by a retina-specific antigen (s-antigen). Purified s-antigen demonstrated rhodopsin kinase activity. Purified rhodopsin kinase showed a single precipitin line with anti s-antigen in immunodiffusion. The molecular weight of the two proteins is essentially the same. These results suggest that rhodopsin kinase and s-antigen are an identical protein. (b) Cyclic nucleotide phosphodiesterase. Cyclic nucleotide phosphodiesterase was found to be localized on the external surface of the disk. Evidence was obtained that rhodopsin kinase quenches the ability of opsin to activate the phosphodiesterase. Thus, a role of the kinase in vivo may be to turn off the level of phosphodiesterase activation. The result suggests that rhodopsin, kinase and phosphodiesterase form a functional⁺⁺ complex in the disk membrane. (c) GTPase (GTP binding protein). When low-Mg⁺⁺ extracts of rod membranes were chromatographed on Sephacryl 200 and Blue Sepharose, GTPase and GTP binding activities were purified and collected in the same fractions. These activities resist to extraction at high Mg⁺⁺ concentrations and can be solubilized only with detergent. Neither the purified enzyme nor purified rhodopsin (in 0.01% Emulphogene) shows GTPase activity both in the light and in the dark. However, when they are incubated together in the light, marked activation of the enzyme

occurred. The result indicates that GTPase activity is manifested as a consequence of the protein-rhodopsin complex formation, and that membranous environment is not essential for the active complex formation. GTPase was found to be associated with the external surface of the disk membrane.

II. Structural features of rhodopsin. (a) The N-terminus of rhodopsin. A sugar-containing glycopeptide was isolated from tryptic digests of rhodopsin and was sequenced: AcetylMet-Asp(CHO)-Gly-Thr-Glu-Gly-Pro, [(CHO)=carbohydrate]. The blocked N-terminal residue is acetylmethionine. (b) The chromophore of rhodopsin. Rhodopsin in the disk fragments spread in a film on a quartz plate shows absorption maxima at 498nm (α) and 340nm (β). Film preparations are advantageous because light scattering effects are minimum. Upon dehydration (<100 μ m Hg) of the film, the α shifted to 390nm. The shift was fully reversible on rehydration. We interpret the spectral change to indicate that the chromophore of rhodopsin is protonated with hydronium ion (H_3O^+) and is deprotonated by dehydration. Upon light irradiation both hydrated rhodopsin and dehydrated rhodopsin produced essentially identical metarhodopsin I (α =480nm) which is protonated but not by hydronium ion. It is suggested that the formation of protonated metarhodopsin I from unprotonated rhodopsin is accompanied by proton translocation from an acidic group of opsin to the retinylidene chromophore. The finding would have a profound impact on the current model of rhodopsin chromophore and on the interpretation of early events in visual transduction.

Significance to Biomedical Research and the Program of the Institute: Experimental uveitis is an autoimmune disease induced by a retina specific soluble antigen. The disease has been investigated extensively as a model for sympathetic ophthalmia. The identification of the antigen with rhodopsin kinase and location of the enzyme on the external surface of the disk membrane will provide new insight into the mechanism of pathogenesis.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium.

Publications:

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Tsunasawa S, Narita K, Shichi H: The blocked N-terminal residue of rhodopsin is acetyl methionine. Biochim Biophys Acta (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00069-03 LVR																									
PERIOD COVERED October 1, 1979, to September 30, 1980																											
TITLE OF PROJECT (80 characters or less) Immune Responses to Ocular Antigens																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Regina Skelly</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Igal Gery</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Robert Nussenblatt</td> <td>Senior Staff</td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td>Ophthalmologist</td> <td>CF</td> <td>NEI</td> </tr> <tr> <td></td> <td>Julia Derr</td> <td>Biologist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Regina Skelly	Staff Fellow	LVR	NEI	Other:	Igal Gery	Visiting Scientist	LVR	NEI		Robert Nussenblatt	Senior Staff					Ophthalmologist	CF	NEI		Julia Derr	Biologist	LVR	NEI
PI:	Regina Skelly	Staff Fellow	LVR	NEI																							
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	Robert Nussenblatt	Senior Staff																									
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	Julia Derr	Biologist	LVR	NEI																							
COOPERATING UNITS (if any) Clinical Branch, NEI																											
LAB/BRANCH Laboratory of Vision Research SECTION Biochemistry																											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205																											
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.1	OTHER: 0.2																									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords) <p> Cellular and humoral immune responses to purified lens crystallins were examined in genetically-defined inbred strains of mice. Primed mice produced small, yet significant, cellular responses to the immunizing crystallins when tested in an in vitro proliferation assay. Crystallins from either mouse (allogeneic) or calf (xenogeneic) were active in the stimulation of these cellular responses. Sera of the immunized mice contained specific antibody activity to the tested crystallins. In other experiments, different strains of mice were immunized against purified retinal S-antigen. Preliminary results indicate that the immunized mice produced both cellular and humoral responses to the S-antigen. No pathologic changes (uveitis) have been observed in the eyes of these animals as of yet. </p>																											

Project Description:

Objectives: Results reported previously (FY 1979) demonstrated that rabbits react well against rabbit lens crystallins by humoral (antibody) but not by cellular immune responses. These studies were extended to the mouse with the aim of determining whether a similar dissociation of the immune response would be found in this species; the availability of genetically-defined strains of mice also affords a means of investigating genetic regulation of these immune responses. Mice with different genetic backgrounds were also used to study immune responses to retinal S-antigen. On-going studies in other species (rat, guinea pig, monkey) demonstrate that immunization with S-antigen produces uveitis along with both cellular and humoral immune responses.

Methods Employed: Mouse or calf lens crystallins (α , β or γ) were purified by chromatography; bovine retinal S-antigen was prepared according to the method of Waeker et al. The antigens were used for immunization after being emulsified in complete Freund's adjuvant. At different time intervals, T lymphocytes from spleen or the regional lymph nodes were tested for specific responsiveness to the antigens in culture, as measured by increased incorporation of thymidine. Serum antibodies to the various antigens were determined by using the ELISA (enzyme-linked-immunosorbent assay) technique.

Major Findings: All three types of lens crystallins (α , β , or γ) from mice stimulated specific antibody responses in all the immunized mice tested. However, only β -crystallins induced detectable cellular response in culture. S-antigen produced both cellular and humoral specific immune responses in all tested mouse strains, but no histologic changes have been observed in the eyes of mice immunized with this antigen, using a number of different immunization protocols.

Significance to Biomedical Research and the Program of the Institute: These studies demonstrate that the mouse may be useful for studying immune responses to ocular components. The investigation of the genetic control of these responses will be feasible. The data obtained so far indicate that the lens crystallins differ markedly in their immunogenicity in the mouse, with β crystallin being superior in induction of cellular immune response. The finding that uveitis is not detected in mice immunized with S-antigen indicate that this species differ from the other tested animals (including rats, guinea pigs, monkeys). The cause for this difference will form the basis for our continued studies.

Proposed course: The cellular immunity to ocular antigens will be further studied in the mouse. The possibility of cloning lymphocytes specifically sensitized to ocular antigens will be tested and if successful, the in vivo and in vitro effects of these antigen-specific clones of cells will be examined. The induction of uveitis by ocular antigens in mice will be tested further by using other immunization protocols, including the use of different adjuvants and vaccines, and other strains of mice.

NEI Research Program: Retinal and Choroidal Diseases--Uveal Tract.
Cataract--Congenital, Metabolic, and Genetic Cataracts.

Publications:

Nussenblatt RB, Gery I, Wacher WB: Experimental auto-immune uveitis: cellular immune responsiveness. Invest Ophthalmol Vis Sci 19:686-690, 1980.

Gery I, Nussenblatt R, BenEzra D: Dissociation between humoral and cellular immune responses to lens antigens. Invest Ophthalmol Vis Sci (in press).

Gery I, Zigler JS Jr, Nussenblatt R: Dissociation between the humoral and cellular immune responses to lens crystallins. Invest Ophthalmol Vis Sci 19(ARVO Suppl):34, 1980.

Nussenblatt R, Gery I, Salinas M, Kuwabara T, Wacker WB: S-antigen induced uveitis in primates and guinea pigs. Fed Proc 39:470, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00105-01 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980 TITLE OF PROJECT (80 characters or less)		
Structure and Composition of Lens Crystallins with Respect to Cataract Development		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. Samuel Zigler, Jr. Other: Jin H. Kinoshita John D. Goosey Deborah A. Carper	Ph.D. Ph.D. M.D. B.A.	Staff Fellow Chief Staff Fellow Biologist
		LVR NEI LVR NEI LVR NEI LVR NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Vision Research SECTION		
Section on Biochemistry INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.3	PROFESSIONAL: 2.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Studies have been undertaken to elucidate the structures of various <u>lens proteins</u> from human lens and from an animal model of cataract called the <u>Philly mouse</u> . In each case marked changes in protein composition occur during <u>cataract development</u> . In addition there are known to be numerous <u>structural modifications</u> to human crystallins which occur during <u>lens aging</u> and cataractogenesis. We have been able to produce a number of these modifications to <u>crystallins</u> including <u>crosslinking</u> of polypeptides, formation of <u>blue fluorophores</u> , <u>pigmentation</u> and formation of <u>heavy molecular weight aggregates</u> , in vitro by using a <u>photosensitized oxidation system</u> . These effects were shown to be mediated by <u>singlet molecular oxygen</u> , a highly reactive excited state of oxygen, produced by the interaction of light and a <u>photosensitizer</u> . We have demonstrated that compounds endogenous to the human lens can act as photosensitizers. These compounds absorb <u>near UV light</u> at wavelengths greater than 330 nm. Light of these wavelengths is abundant in the lens. The photosensitizers appear to be bound to the crystallins, especially the <u>insoluble protein</u> , and accumulate with age. We believe that photosensitized oxidation mediated by singlet oxygen could be playing a major role in vivo in human lens aging and cataractogenesis.		

Project Description:

Objectives: Lens crystallins are the major structural elements of the vertebrate lens and are believed to play a major role in the development of some types of human cataract. The aim of this study is to determine how the crystallins change during normal aging and cataractogenesis. Approaches to this problem include isolation and characterization of proteins from normal and cataractous human lenses, the qualitative and quantitative analysis of crystallins during cataract development in animal model systems, and studies in vitro using a model system based on the hypothesis that photochemical damage to lens crystallin is an important factor in human lens aging and senile cataract development.

Methods Employed: Various proteins from human and mouse lenses, both normal and cataractous, have been isolated. They have been analyzed using column chromatography, gel electrophoresis, isoelectric focusing and immunochemical techniques. Photochemical methods involving visible and near ultraviolet irradiation with various exogenous and endogenous photosensitizers have been utilized with human crystallin.

Major Findings: Studies on human lens crystallins have been continued with emphasis on possible changes in the relative composition of the crystallins from various types of cataracts. The α , β , and γ crystallin from normal human lens had previously been isolated and specific antibodies prepared to each of them. Quantitative immunochemical techniques were employed using these antibodies to determine the concentrations of the various crystallins in normal lenses and in age-matched cataracts which had been classified by the CCRG criteria. Normal lenses older than 35 years had about 45% α -crystallin, 50% β -crystallins and 5% γ -crystallin. Early cataracts showed only slight changes. Studies on brunescant cataracts indicate significant changes in crystallin composition, however the results also suggest that complexes between different crystallins are forming which makes unequivocal quantitation difficult. Both quantitative immunoelectrophoresis and radio immunoassay (RIA), have been used in these studies. The RIA was applied in particular to the β -crystallin in hopes of being able to quantitate β_1 , β_2 , and β_3 separately. By immunodiffusion all three classes of β -crystallins give reactions of identity for the major antigen present. The RIA however did allow us to quantitate β_2 because this particular class contains a unique minor antigen which the highly specific competitive binding type analysis of the RIA can separate from the antigens common to all 3 β -crystallin classes.

A major hypothesis for the etiology of human senile cataract, particularly nuclear cataracts, has held that near UV light is an important factor. Most investigators have considered only the possibility of direct effects of UV on aromatic amino acids in the lens. We have recently initiated studies on the possibility that the near UV effects are mediated by photosensitizing compounds in the lens. The human lens is known to contain chromophores which absorb in the near UV and which could potentially act as photosensitizers. In vitro using known visible and UV photosensitizers we

have been able to produce a number of modifications to lens crystallins which closely parallel documented changes occurring during aging and cataractogenesis in human lens. These modifications include the production of covalent crosslinks between crystallin polypeptides, a characteristic blue fluorescence, pigmentation of the proteins, and aggregation to form heavy molecular weight aggregates. These changes in our system are absolutely dependent upon the presence of oxygen. Since photosensitized oxidations generally work via one or more of the activated forms of oxygen we utilized specific scavengers of these molecules to determine which species is producing the observed effects. Such studies indicated that only singlet oxygen was playing a major role in our system. This finding has been confirmed by using photophysically produced singlet oxygen, thus eliminating the possibility that direct interaction between the crystallins and the photosensitizer could cause the protein modification. We propose therefore that singlet oxygen may play an important role in the changes in crystallin which occur during human lens aging and cataractogenesis. Such a mechanism would require only light of the appropriate wavelength, oxygen and a photosensitizer. The lens receives plenty of light and contains a low, but probably sufficient concentration of oxygen. We have recently established that compounds known to exist in the lens are good photosensitizers in vitro. These compounds are near UV absorbing compounds, including kynurenine derivatives, which apparently are produced from aromatic amino acids. We also have shown that the pigmented insoluble protein from brunescant cataracts contains photosensitizing compounds. Present evidence suggests that these protein bound photosensitizers could be producing singlet oxygen in the lens in vivo. Endogenous antioxidants such as glutathione would act as protective agents. The fact that glutathione concentration is reduced in the lens nucleus where the concentration of the sensitizing compounds is highest fits with the localization of the nuclear, pigmented cataracts.

In collaboration with Dr. Joseph Horwitz of UCLA we have continued studies on the major intrinsic protein of lens fiber cell membranes. We had previously demonstrated the presence in human lens of a second major intrinsic membrane protein with molecular weight of 22,000 as well as the 26,000 dalton polypeptide characteristic of other vertebrates. Since the 22,000 dalton species increased with age, especially in the lens nucleus, it was postulated that it was the product of proteolytic cleavage of the 26,000 dalton species. To test this idea an antibody was prepared to highly purified bovine 26,000 dalton membrane polypeptide. This highly specific antibody was shown to cross-react with both human intrinsic polypeptides indicating a close structural relationship between the two. Furthermore, while reaction of identity was found on immunodiffusion between the 26,000 dalton species from bovine and human lens, only partial identity was found between the 26,000 species and the 22,000 dalton chain. This is consistent with loss of one or more determinants of the 26,000 in formation of the 22,000 and strongly supports the hypothesis that cleavage of the 26,000 dalton species leads to the 22,000 dalton polypeptide.

Studies have also been conducted on changes in lens crystallins which occur in a new hereditary cataract of mice, called the Philly cataract. Quantitative rocket immunoelectrophoresis revealed that Philly mouse lens before development of visible cataract has a crystallin composition quite similar to normal mice with about half the soluble crystallin being γ -crystallin. After the cataract forms the content of γ -crystallin drops to 12% of total soluble protein and β -crystallin also decreases to a lesser extent. Some of the lost soluble protein leaks from the lens since both γ and β -crystallins were detected in the aqueous of Philly mice with cataracts. There is also an increase in insoluble protein in the cataracts accounting for some of the loss of soluble protein. Interestingly there are structural differences in some of the Philly crystallins when compared to those of normal mice by isoelectric focussing. These changes are present even before the cataract starts developing and thus could represent primary events in the etiology of this cataract in contrast to the situation in the Nakano cataract where all crystallin changes appear to be secondary to cataract development.

Significance to Biomedical Research and the Program of the Institute:

A variety of changes in crystallins are well-documented to occur in the development of human cataracts. The various approaches outlined above are attempts at determining the mechanism of some of these changes as well as the possible role they may play in cataract development.

Proposed Course: Strong emphasis will be placed on the photochemical studies with lens crystallin since work to date has been so strongly suggestive that photosensitized oxidation may account for several well-known modifications to crystallin in old or cataractous human lens. Efforts are underway to demonstrate these effects in whole lenses in organ culture. We also must demonstrate that the relatively low oxygen levels present in vivo are sufficient for photodynamic effects mediated by the various endogenous photosensitizers. Efforts will also be made to determine which amino acid residues are being destroyed or altered in our system and which residues are involved in the formation of covalent crosslinks. In conjunction with these studies will be further structural studies on isolated crystallins from cataracts, especially brunescient lenses which are the cataracts most likely to have a strongly photochemical etiology.

NEI Research Program: Cataract--Senile or Degenerative Cataract

Publications:

Zigler JS Jr, Horwitz J, Kinoshita JH: Human β -crystallin I. Comparative studies on the β_1 , β_2 and β_3 crystallin. Exp Eye Res (in press).

Zigler JS Jr, Carper DA, Russell P, Kinoshita JH: Analysis of immunochemical properties of human β -crystallin by radioimmunoassay. Exp Eye Res (in press).

Zigler JS Jr, Horwitz J, Kinoshita JH: Studies on the low molecular weight proteins of human lens. Exp Eye Res (in press).

Russell P, Uga S, Zigler JS Jr, Kaiser-Kupfer M, Kuwabara T: Studies using human lens from a family displaying hereditary congenital cataracts. Proceedings of Ocular Tissue Culture Symposium. (in press).

Goosey JD, Zigler JS Jr, Kinoshita JH: Cross-linking of lens crystallins in a photodynamic system: A singlet oxygen mediated process. Science 208:1278-1280, 1980.

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Gery IB, Zigler JS Jr, Nussenblatt R: Dissociation between the humoral and cellular immune responses to lens crystallins. Invest Ophthalmol Vis Sci 19(ARVO Suppl):34, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00177-05 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980.		
TITLE OF PROJECT (80 characters or less) Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Peggy Zelenka	Ph.D Geneticist LVR NEI
Other:	Gloria Chepko	Ph.D. Staff Fellow LVR NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Experimental Embryology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p> This project seeks to determine whether the regulation of <u>lens fiber differentiation</u> and maturation is associated with alterations in the <u>plasma membrane</u>. To this end, the principal <u>lipid</u> and <u>protein</u> components of embryonic and adult chicken lens membranes are being identified, and their metabolism is being investigated. Because of the known involvement of <u>phosphatidylinositol</u> (PI) turnover and <u>phosphatidylethanolamine</u> (PE) methylation in regulatory mechanisms of various other cell types, the initial stages of this study have focused on lens <u>phospholipid</u> metabolism. <u>Computer modeling</u> of the kinetics of ³²P incorporation into lens phospholipids in vivo is employed to determine the rates of synthesis and degradation of individual phospholipids. This approach is also being applied to the study of phospholipid metabolism in differentiating explants of embryonic chick lens epithelia in <u>organ culture</u>, thus allowing the possible relationships between phospholipid metabolism and differentiation to be studied under controlled conditions. </p>		

Project Description:

Objectives: The objectives of this project are: (a) to characterize the principal lipid and protein components of plasma membranes from embryonic chick lens fibers and lens epithelial cells; (b) to determine whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in membrane composition; (c) to learn whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in the metabolism of lens plasma membranes; and (d) to establish the functional significance of any changes in membrane composition or metabolism.

Methods Employed: ^{32}P -labeled phospholipids for in vivo studies of phospholipid metabolism are obtained by injecting isotope into six-day-old chick embryos via the chorioallantoic circulation. Lens fibers and epithelia are isolated by microdissection of the embryos and, in some cases, lens epithelia are further dissected into central and peripheral regions. Phospholipids are extracted and separated by thin layer chromatography; radioactivity is determined either by scintillation counting or by autoradiography. Rates of synthesis and degradation of individual phospholipids are determined by mathematical modeling, using a single-compartmental model. Precursors of phospholipid biosynthesis are isolated by thin-layer chromatography after in vitro or in vivo labeling with ^{32}P .

In vitro studies of lens phospholipid metabolism employ cultured explants of lens epithelia from 6-day-old or 19-day-old embryonic chicks. Various culture conditions are used to maintain the cells in an epithelial state or to permit their differentiation into lens fibers. Phospholipids are labeled with ^{32}P -orthophosphate, ^3H -glycerol, ^3H -methionine, or ^3H -labeled fatty acids. Drugs which interfere with phospholipid metabolism are added to the culture medium to test their effect on differentiation. Labeled phospholipids are analysed by thin layer chromatography and scintillation counting.

Polyphosphoinositides from lenses of mouse, rat, and chicken are isolated by affinity chromatography on glass bead columns containing covalently bound neomycin. Phosphorous analysis is then used to determine the amount of polyphosphoinositide present.

Fatty acids of lens fibers and epithelia are analysed by gas-liquid chromatography. Fatty acid methyl esters are formed from unfractionated phospholipid extracts or from specific phospholipids isolated by two-dimensional thin layer chromatography.

Major Findings: The concentration of phosphorylcholine (PhCh) and phosphorylethanolamine (PhEt) in 6-day-old embryonic chick lens epithelia and fiber masses was measured as a preliminary step in determining the specific activities of these compounds after in vivo injection of ^{32}P . The lens epithelium contained 20 pmoles PhCh and 130 pmoles PhEt, while the fiber mass contained 140 pmoles PhCh and 720 pmoles PhEt. Because of these high concentrations of PhCh and PhEt in the embryonic chick lenses, changes in the

specific activities of these compounds after in vivo injection of isotope are very slow. The low specific activity of these precursor pools can mask high rates of synthesis and degradation of the phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

Analysis of the in vivo labeling data for PC and PE revealed that both phospholipids are rapidly degraded in the epithelia ($t_{1/2}$ of PC=8hrs; $t_{1/2}$ of PE=10hrs) but are stable in the lens fibers ($t_{1/2}$ of PC=22 hrs; $t_{1/2}$ of PE=46hrs). Thus all three phospholipids that have been analysed in detail (PI, PC, and PE) show rapid rates of turnover in the lens epithelium but not in the lens fibers.

In some experiments the central and peripheral regions of lens epithelia were separated by microdissection after in vivo labeling with ^{32}P . Preliminary results indicate that the rapidly metabolised phospholipids are found primarily in the peripheral epithelium. This region contains cells that are in the initial stages of lens fiber formation.

The results of the in vivo experiments have been confirmed and extended by in vitro studies. Epithelial explants in early stages of differentiation contain two pools of PI and PC that can be distinguished by labeling kinetics. One pool has an apparent half-life of 4-5 hrs, while the other appears to be stable, with a half-life greater than 30 hrs. There was no difference in the metabolism of the glycerol backbone and the phosphate-containing headgroup of either phospholipid.

Incubating the lens epithelial explants in the presence of 10^{-6}M arachidonic acid greatly increased the size of the rapidly degraded pool of PI. Oleic acid did not produce this effect. This result suggests that the rapidly metabolized pool of PI may be the arachidonate-containing PI.

Significance to Biomedical Research and the Program of the Institute:

The plasma membranes of lens cells appear to play important roles in normal development and function of the lens. In addition, they are centrally involved in the genesis and development of several varieties of lens cataract. Despite the widely recognized and important functions of these membranes, work on their composition, turnover, and development has begun only recently. This project focuses on changes in lens cell membranes which are associated with lens fiber differentiation. These results should have broad application in understanding normal lens differentiation and morphogenesis and in attempts to establish etiologies for several types of cataract.

Proposed Course: This project will be continued. Additional experiments will be undertaken to characterize the changes in phospholipid metabolism that accompany lens fiber formation and to ascertain their biological significance.

- a) The metabolic fate of phospholipids undergoing rapid turnover in the lens epithelium will be investigated. Experiments will be done to test for methylation of PE to PC, phosphorylation of PI to poly-

phosphoinositides, and deacylation of each of the phospholipids to the corresponding "lyso-" derivatives.

- b) The action of arachidonic acid on cultured explants will be investigated. Epithelial explants will be tested for their ability to synthesize prostaglandins during the initial stages of lens fiber formation, and the effects of agents which block prostaglandin biosynthesis will be observed. The arachidonic acid content of lens fibers and epithelia of 6-day-old embryonic chicks will be determined by gas chromatography. The results will be compared with those obtained from lenses of other species and other developmental ages.
- c) Specific inhibitors of enzymes involved in phospholipid biosynthesis will be tested for their effects on in vitro lens fiber formation. Conversely, substances known to inhibit lens fiber formation will be tested for effects on phospholipid metabolism.

NEI Research Program: Cataract--The Normal Lens

Publications:

Zelenka P: Changes in phosphatidylinositol metabolism during differentiation of lens epithelial cells into lens fiber cells in the embryonic chick. J Biol Chem 255:1296-1300, 1980.

Zelenka PS: Phospholipid metabolism in lens epithelia and fiber masses of 6-day-old embryonic chicks. Invest Ophthalmol Vis Sci 19(ARVO Suppl): 53, 1980.

Chepko GJ, Zelenka PS: Alterations in phospholipid metabolism associated with in vitro differentiation of embryonic chick lens epithelia. Invest Ophthalmol Vis Sci 19(ARVO Suppl):149, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00032-04 LVR															
PERIOD COVERED October 1, 1979, to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Effects of Illumination on Vitamin A Deficient Retinas																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Louvenia Carter-Dawson</td> <td style="width: 33%;">Ph.D. Staff Fellow</td> <td style="width: 33%;">LVR NEI</td> </tr> <tr> <td>Other: Toichiro Kuwabara</td> <td>M.D. Head, Section on</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Experimental Pathology</td> <td></td> </tr> <tr> <td>John G. Bieri</td> <td>Ph.D. Chief, Section on</td> <td></td> </tr> <tr> <td></td> <td>Nutritional Biochemistry</td> <td>LNE NIAMDD</td> </tr> </table>			PI: Louvenia Carter-Dawson	Ph.D. Staff Fellow	LVR NEI	Other: Toichiro Kuwabara	M.D. Head, Section on	LVR NEI		Experimental Pathology		John G. Bieri	Ph.D. Chief, Section on			Nutritional Biochemistry	LNE NIAMDD
PI: Louvenia Carter-Dawson	Ph.D. Staff Fellow	LVR NEI															
Other: Toichiro Kuwabara	M.D. Head, Section on	LVR NEI															
	Experimental Pathology																
John G. Bieri	Ph.D. Chief, Section on																
	Nutritional Biochemistry	LNE NIAMDD															
COOPERATING UNITS (if any) Laboratory of Nutrition and Endocrinology, NIAMDD																	
LAB/BRANCH Laboratory of Vision Research																	
SECTION Section on Experimental Pathology																	
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																	
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) <u>Vitamin A (retinol)</u> is essential for the maintenance of <u>retinal photoreceptor cell</u> structure and function. In the absence of this vitamin, photoreceptor cells <u>degenerate</u> . The rate of degeneration is fastest in the <u>inferior hemisphere</u> of the eye. Although light accelerates the rate of cell loss, the regional difference in rate of degeneration occurs independent of environmental lighting. Perhaps regional differences in rate of cell loss is related to a diversity in metabolism and/or stores of retinol or the interrelationship between photoreceptor cells and pigment epithelium.																	

Project Description:

Objectives: In dim illumination (1.5-2 foot-candles) photoreceptor cells degenerate at a faster rate in the inferior retinal hemisphere of vitamin A deficient rats. A similar pattern of photoreceptor cell loss is seen after dark rearing rats with a genetic lesion. It is unclear whether the dim illumination induced the regional difference in the vitamin A deficient retinas. Thus we designed this study to examine the influence of environmental lighting on regional degeneration.

Methods Employed: Albino rats fed a retinol deficient or retinol adequate diet were placed in one of three environmental lighting conditions. Rats in one group, were reared in cyclic light (12 hours light - 12 hours dark) of 10 foot-candles through 22 weeks. Rats in group II were reared in cyclic light (10 foot-candles) for seven weeks followed by 22 weeks in darkness. In group III, rats were maintained in darkness through 29 weeks. The number of photoreceptor cells present in seven consecutive 90 μ m segments of posterior retina was recorded from the inferior and superior hemispheres at 9, 13 and 29 weeks, and the mean and standard error of the mean were determined.

Major Findings: Neither light nor darkness induces regional degeneration in vitamin A deficient rat retinas. At 29 weeks on the vitamin A deficient diets, rats reared in cyclic light had lost 39 percent of their photoreceptor cells in the inferior hemisphere but only 15 percent in the superior hemisphere. Rearing the rats in cyclic light for seven weeks followed by 22 weeks in darkness or in complete darkness for 29 weeks slowed the rate of photoreceptor loss. In combined light and darkness 24 percent were lost in the inferior hemisphere and 12 percent in the superior hemisphere. Rats reared in complete darkness for 29 weeks lost 23 percent of the photoreceptor cells in the inferior hemisphere and 11 percent in the superior hemisphere. Photoreceptor cells degenerate faster in the inferior retinal hemisphere of vitamin A deficient (retinol) rats independent of the lighting conditions; however, the rate of degeneration is accelerated by light.

Significance to Biomedical Research and the Program of the Institute: Study of the retina in vitamin A deficiency provides additional information on the role of this vitamin in the maintenance of normal structure and function. Information obtained from such a study can be useful in identifying human disorders which may involve defects associated with uptake, storage and/or utilization of this vitamin in the retina.

Proposed Course: Photoreceptor cells degenerate at a different rate in the superior and inferior retinal hemispheres. The basis for the different rates of degeneration is not understood. However several possibilities can be considered to explain this phenomenon. First, the metabolism and/or amount of retinol stored in each region may differ. Second, the ratio of outer segment number per pigment epithelial cell may differ. These possibilities will be investigated.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells
and Pigment Epithelium

Publications:

Carter-Dawson L, Tanaka M, Kuwabara T, Bieri JG: Early corneal changes in vitamin A deficient rats. Exp Eye Res 30:261-268, 1980

Carter-Dawson L, Kuwabara T, Bieri JG: Effects of dark-rearing on vitamin A deficient retinas. Invest Ophthalmol Vis Sci 19 (ARVO suppl):190, 1980.

Currier CA, Newsome DA, Carter-Dawson L, Harne LC, Brown KS: Inherited severe mouse xerophthalmia resists vitamin A treatment. Invest Ophthalmol Vis Sci 19(ARVO) suppl):26, 1980.

Datiles M, Fukui HN, Kinoshita JH, Fukushi S, Carter-Dawson L: Corneal reepithelialization in the galactosemic rat. Invest Ophthalmol Vis Sci 19(ARVO) suppl):75, 1980.

Carter-Dawson L, Kuwabara T, Bieri JG: Effects of moderate-intensity light on vitamin A deficient rat retinas. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00129-08 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Anatomical and Pathological Studies of Ocular Tissues		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Toichiro Kuwabara Other: Yasumichi Yajima Yujiro Ishikawa Shigeru Uga Carole Latker	M.D. M.D. M.D. M.D. Ph.D.	Head, Section on Experimental Pathology Visiting Scientist Visiting Scientist Visiting Scientist Guest Worker LVR NEI LVR NEI LVR NEI LVR NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Experimental Pathology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5.0	PROFESSIONAL: 5.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Histopathological studies</u> were conducted on numerous human and animal eyes by transmission and scanning <u>electron microscopy</u> , <u>histochemistry</u> and histological sectioning. Studies conducted on the cornea, lens, and aqueous humor are all related to certain clinical problems.		

Project Description:

Objectives: Clarify the normal structure and function of each cell of the eye in order to add to understanding of the pathophysiology of various eye diseases. Also, to study systematically the eye with naturally occurring diseases to elucidate further the pathogenesis involved.

Methods Employed: A large number of clinicopathological specimens sent to this laboratory from various eye research centers throughout the world were studied. Details on individual experiments on animals are described under Major Findings.

These eye tissues were fixed in glutaraldehyde solution and processed for transmission and scanning electron microscopy. Depending on specific diseases, various types of histochemical reactions were applied on cryo-, frozen, paraffin and plastic sections.

Major Findings: In the past, we have been involved with studies of the wound healing of the cornea. Recently we turned our attention to the process of wound healing in the lens. The healing mechanism of a small wound produced by pricking the anterior center of a Swiss Webster mouse lens with a glass needle was studied histologically. The first response to the injury was the oozing out of the superficial cortical substance which later began to degenerate. The epithelial cells slowly began to proliferate and the defective area was covered with many cells by the fifth day. Also, proliferation of the epithelial cells near the wound was found to occur during incubation of the wounded lenses in a tissue culture medium for 24 hours. Whole mount preparations of the epithelium revealed active mitotic activity in the area adjacent to the wound for about a week.

The proliferated cells overlying the wound were spindle in shape and contained abundant filaments and rough endoplasmic reticulum. The cells at the bottom of the wound formed a continuous chain by the seventh day. The proliferating cells formed a meshwork of the tissue consisting of basal lamina, filamentous substances and collagen fibers by 30 days post-injury. The cells in this connective tissue gradually disappeared and the wound was covered with the irregularly arranged epithelial cells by the fourth month. It was not until this period that the thin capsule was formed on the anterior surface of these epithelial cells. No sign of lens fiber formation was noted in the wounded area. It is noteworthy that the healing mechanism in the mouse lens is relatively slow.

Another area of interest is the postnatal changes of the hyaloid vascular system. Postnatal changes in the vascular pattern of the regressing hyaloid system in the albino rat were investigated. Scanning electron microscopy (SEM) demonstrated two groups of hyaloid vessels radiating from the optic disc. An inner, central group extended to the posterior lens pole and an outer, peripheral group to the lens equator. On the lens surface these

vascular channels formed a complex interlacing pattern, frequently branched, and interconnected by short vessels. Large cells with ruffled membranes were seen in the vicinity of the outer surfaces of these vessels and the lens capsule. The wall of the hyaloid capillaries adjacent to the lens was in close association with the lens capsule.

SEM demonstrated that as regression began the short connecting vessels decreased in size or disappeared, leaving long straight vascular channels. Later, some of the vessels became thin or disappeared while adjacent larger ones persisted. LM revealed a number of capillaries on the lens surface rounded up and pulled away from the capsule. In some capillaries, the walls although collapsed appeared normal while in others, which were patent, the walls became acellular. Many vessels seemed to be functional several weeks after birth while others showed deleterious changes early in the period. This study suggests that several modes of involution may occur resulting in different rates of regression in a given area, thereby slowly decreasing vascular perfusion to the developing lens. The close association of the large cells with the vessels may indicate a functional interaction.

Significance to Biomedical Research and the Program of the Institute:

The staff of this section is able to pursue a multidisciplinary study to attack problems which are directly related to clinical ophthalmology. Further clarification of the normal and abnormal structure and function of ocular tissues and cells is a significant part of eye research.

Proposed Course: Similar projects are actively ongoing and will be continued in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders/Inflammatory Disorders/Uveal Tract; Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders/Corneal Transplantation and Stroma Injury and Repair/Tumors and Other Lid, Conjunctival, and Orbital Problems; Cataract--The Normal Lens/Cataract Induced by Drugs and Radiation and Occurring Secondary to Other Eye Disorders; Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma/Secondary Glaucomas)

Publications:

Kuwabara T: Species differences in the pigment epithelium, in Zinn K, Marmor M (eds): The retinal pigment epithelium, Cambridge, Harvard Press, 1979, pp 58-82.

Kuwabara T: Photic and photo-thermal effects on the retinal pigment epithelium, in Zinn K, Marmor M (eds): The retinal pigment epithelium, Cambridge, Harvard.

Kuwabara T: Age-related changes of the eye, in Han SS, Coons DH (eds). Special senses in aging, Ann Arbor, Institute of Gerontology, U of Mich Press, 1979, pp 46-78.

Chu FC, Kuwabara T, Cogan DG, Schaefer EJ, Brewer HB Jr: Ocular manifestations of familial high-density lipoprotein deficiency (Tangier Disease). Arch Ophthalmol 97:1926-1928, 1979.

Tanaka M, Russell P, Smith S, Uga S, Kuwabara T, Kinoshita JH: Membrane alterations during cataract development in the nakano mouse lens. Invest Ophthalmol Vis Sci 19:619-629, 1980.

Uga S, Yajima Y, Kuwabara T: Wound healing mechanism in the mouse lens. Invest Ophthalmol Vis Sci 19(ARVO Suppl):117, 1980.

Uga S, Kador P, Kuwabara T: Cytological study of philly mouse cataract. Exp Eye Res 30:79-92, 1980.

Latker CH, Kuwabara T: Postnatal changes of the hyaloid vascular system in the rat. Invest Ophthalmol Vis Sci 19(ARVO Suppl):35, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00149-07 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Ultrastructure and Function of the Pigment Cells of the Eye		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: W. Gerald Robison, Jr. Other: Toichiro Kuwabara John G. Bieri Stephen M. Sykes	Ph.D. Geneticist/Cell Biologist M.D. Head, Section on Experimental Pathology Ph.D. Chief, Section on Nutritional Biochemistry M.S. Biologist, Experimental Studies Branch, Division of Biological Effects	LVR NEI LVR NEI LNE NIAMDD FDA
COOPERATING UNITS (if any) Laboratory of Nutrition and Endocrinology, NIAMDD Bureau of Radiological Health, FDA		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Experimental Pathology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p> The availability of <u>vitamin A (retinol)</u> and the availability of discarded <u>rod outer segment membrane</u> in the <u>retina</u> were varied independently in order to determine which had the greater influence on the accumulation of <u>lipofuscin (aging pigment)</u> in the <u>retinal pigment epithelium</u> (RPE). Rats missing all rod outer segments due to <u>heredity</u>, and rats missing most of their rod outer segments due to <u>light damage</u> were compared to normal rats on diets either deficient or adequate in vitamin A. Within each diet group, the RPE of rats which lacked photoreceptor outer segments exhibited only slightly less lipofuscin than did the RPE of normal rats. Between diet groups, the lipofuscin was always markedly less in <u>vitamin A-deficient retinas</u>. Even in retinas with photoreceptors present, <u>vitamin A deprivation</u> resulted in greatly reduced amounts of lipofuscin. In conclusion, the presence of outer segment membranes has some influence on lipofuscin accumulation, but retinol makes a much greater contribution. Perhaps retinol, which is known to be highly concentrated in the RPE and to exhibit dynamic turnover in the retina is involved directly in lipofuscin formation. </p>		

Project Description:

Objectives: To study the associations of dietary vitamin A and the intracellular breakdown of membrane lipids with the accumulation of aging pigment in the retina as they relate to the roles of the pigment epithelial cells in the maintenance of photoreceptor cells. This is part of our continued effort to examine what specific functions of the pigment epithelial cells are altered or lacking under various experimental and pathological conditions that might influence their ability to provide proper maintenance of the visual apparatus.

Methods Employed: We designed an experiment to determine the possible dependence of retinal lipofuscin accumulation on the retinol as well as on the other lipids present in the retina using normal rats, and rats which lacked photoreceptor outer segments due to heredity or light damage. Weanling female Royal College of Surgeons rats with dystrophic retinas (RCS-p/p, rdy/rdy) and congenic control rats with normal retinas (RCS-p/p, +/+) were separated into groups and fed purified diets adequate or deficient in vitamin A. After four weeks, half the rats with normal retinas were exposed to continuous illumination of 400 foot-candles for 45 days to eliminate almost all their photoreceptor cells by retinal light damage. After 24 weeks and 36 weeks of diet, the retinas were examined for lipofuscin-specific auto-fluorescence using fluorescence microscopy of frozen sections, and were analyzed for numbers of lipofuscin granules using light and electron microscopy.

Major Findings: Retinas with photoreceptor cells present exhibited somewhat more accumulation of lipofuscin in the pigment epithelium than did retinas without photoreceptors. Therefore, some of the lipids of photoreceptor outer segment membranes which are regularly ingested and digested by pigment epithelial cells probably are incorporated into lipofuscin granules after becoming highly oxidized. Vitamin A-deficient retinas always exhibited markedly less accumulation of lipofuscin in the pigment epithelium, suggesting that derivatives of vitamin A must contribute significantly to the content of lipofuscin granules. Vitamin A probably becomes oxidized at its conjugated double bonds, combines with some protein, and becomes an autofluorescent component of lipofuscin granules. Therefore, not only is vitamin A important in the maintenance of the retina, but it must be considered also as a source of some of the undigestible intracellular waste products which accumulate in the retina with age and disease.

Significance to Biomedical Research and the Program of the Institute: In the retina, the pigment epithelium is the only layer which accumulates striking amounts of lipofuscin with age, disease, or antioxidant deficiency. Large quantities of lipid-rich disc membranes discarded from rod outer segments are ingested and rapidly digested by the pigment epithelium everyday. The accumulation of lipofuscin in the pigment epithelial cells has been attributed to this dynamic turnover of the polyunsaturated photoreceptor membrane lipids which are exceptionally susceptible to autoxidation. However,

vitamin A (retinol) is also a lipid which is sensitive to autoxidation, is also involved in the dynamic turnover of rod outer segment membranes, and is present in rather high concentrations in the pigment epithelium. Therefore, in spite of the popular theory that lipofuscin formation results almost exclusively from the oxidation of polyunsaturated fatty acids, it is not surprising that our results in the retina indicate a major contribution by oxidation products of vitamin A. These results broaden our previous findings on the interrelationships that exist between the roles of vitamins A and E in the retina. Lack of vitamin A or lack of photoreceptor outer segments decreases the amount of lipofuscin formation by eliminating oxidizable substrate, whereas lack of vitamin E increases the oxidation of all substrates available, both polyunsaturated fatty acids and vitamin A. Such studies on the roles of vitamins A and E in the retina should contribute to our understanding of the ocular effects of overdose, of malnutrition, of diseases involving limited lipid absorption, and of nervous system diseases and other disorders where aging pigment accumulates prematurely.

Proposed Course: Rats with and without photoreceptor outer segments will be given chronic high doses of vitamin A in order to determine if more waste products (lipofuscin granules) are formed in these cases. Different levels of the antioxidant vitamin E will be provided and carefully maintained. Also, studies will continue on rats deprived of vitamin E and maintained under various light cycles and intensities to see how light damage to the retina is altered without the protective influence of α -tocopherol.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Bieri JG, Tolliver TJ, Robison WG Jr, Kuwabara T: Lipofuscin in vitamin E deficiency and the possible role of retinol. Lipids 15: 10-13, 1980.

Chu F, Ishikawa Y, Robison WG Jr, Kuwabara T, Cogan DG: Fine structural study of lacrimal gland carcinoma. Invest Ophthalmol Vis Sci 19 (ARVO suppl):122, 1980.

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Sykes SM, Clarke BJ, Robison WG Jr: The role of rhodopsin in light-induced retinal damage. Invest Ophthalmol Vis Sci 19 (ARVO suppl):190, 1980.

Robison WG Jr, Kuwabara T, Bieri JG: Deficiencies of vitamin E and A in the rat: Retinal damage and lipofuscin accumulation. Invest Ophthalmol Vis Sci 19:1030-1037, 1980.

Russell P, Robison WG Jr, Kinoshita JH: A new method for isolating the main intrinsic membrane polypeptide from lens. Exp Eye Res (in press).

Sykes SM, Robison WG Jr, Bieri JG: Retinal damage by cyclic light and the effect of vitamin E. Symposium on Biological Effects and Measurement of Light Sources, in Hazzard D (ed): DHHS (FDA) Publication, Washington DC (in press).

Sykes SM, Robison WG Jr, Waxler M, Kuwabara T: Damage to the monkey retina by broad spectrum fluorescent light. Invest Ophthalmol Vis Sci (in press).

Robison WG Jr, Kuwabara T, Zwaan J: The mouse in biomedical research, in Foster HL, Small JP, Fox J (eds): Eye Research, Chapter 62. New York, Academic Press, Inc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00005-08 LVR												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Electrophysiology, Morphology, and Structure of Mammalian and Avian Retinas														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Ralph Nelson</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 33%;">Physiologist</td> <td style="width: 19%;">LVR NEI</td> </tr> <tr> <td>Other: Avery Nelson</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LVR NEI</td> </tr> <tr> <td>Andrew Mariani</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR NEI</td> </tr> </table>			PI: Ralph Nelson	Ph.D.	Physiologist	LVR NEI	Other: Avery Nelson	Ph.D.	Senior Staff Fellow	LVR NEI	Andrew Mariani	Ph.D.	Staff Fellow	LVR NEI
PI: Ralph Nelson	Ph.D.	Physiologist	LVR NEI											
Other: Avery Nelson	Ph.D.	Senior Staff Fellow	LVR NEI											
Andrew Mariani	Ph.D.	Staff Fellow	LVR NEI											
COOPERATING UNITS (if any) Department of Physiology, University of Utah, Salt Lake City; Max-Planck Institut fur Physiologische und Klinische Forschung, Bad Nauheim, F.R.G.; Depart- ment of Ophthalmology, Columbia University College of Physicians and Surgeons, New York.														
LAB/BRANCH Laboratory of Vision Research														
SECTION Section on Neurophysiology														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 2.75	PROFESSIONAL: 2.75	OTHER:												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) We study <u>intracellularly</u> the <u>physiology</u> of <u>single retinal neurons</u> in cats and pigeons. Individual neurons stained through the <u>microelectrode</u> with <u>horse-radish peroxidase</u> (HRP), are classified anatomically after physiological recordings. Using the resolution of the <u>electron microscope</u> , we can then identify <u>synaptic contacts</u> made between the stained cells, which have electron opaque profiles, and other retinal neurons such as <u>photoreceptor</u> , <u>horizontal</u> , <u>bipolar</u> , <u>amacrine</u> and <u>ganglion cells</u> . The response properties of <u>flat and invaginating cone bipolar cells</u> of the cat and the horizontal cells of the pigeon have been identified. The morphology of individual neurons in <u>monkey</u> , <u>cat</u> and <u>pigeon retinas</u> is also studied at the <u>light and electron microscopic</u> levels using <u>Golgi impregnation</u> . The connections between primate bipolar cells and cones have been so observed. A new type of <u>invaginating bipolar</u> , the 'diffuse' variety, has been identified. Primate retinas fixed for <u>ultra-structure</u> demonstrate <u>contacts between photoreceptor terminals</u> and their variation in density and type with retinal location. Structural differences in <u>cone conducting fibers</u> and <u>pedicles</u> occur between <u>foveolar</u> and <u>extra-foveolar</u> cones.														

Project Description:

Objectives: To understand the functional, structural, and ultrastructural organization of mammalian and avian retinas, to discover the synaptic interconnections among neurons and the functional pathways between them, to examine synaptic ultrastructure and observe the modifications produced by stimulation and disease states of the retina.

Methods Employed: We use Golgi impregnation to study the morphology of retinal neurons, to classify them, and to gain insight into function in the retinas of cat, monkey, and pigeon. Electron microscopy of Golgi impregnated cells of the outer plexiform layer provides information about connections with photoreceptors; use of Rall's modification to the cable equations allows the inference, from structural measurements, of signal propagation within cells. Electron microscopy of photoreceptor terminals indicates their interconnections.

We characterize the response properties of neurons in the cat and pigeon retinas by intracellular recording of their transmembrane potentials and extracellular electroretinographic (ERG) recording during photic stimulation. Viable retina-eyecup preparations are maintained in vivo through perfusion of the ophthalmic arteries and retinal surface with synthetic media. HRP, injected into neurons through the electrodes, fills their axons and dendrites, and after incubation with appropriate reagents, the morphology of individual, physiologically studied neurons is revealed in the light and electron microscope. In the light microscope cells are drawn and classified according to analogy with their Golgi counterparts; in the electron microscope the synapses forming the input and output of the unit can be identified by the ultrastructural features of the neighboring unstained processes. Thus, the synaptic relationship of the physiologically studied cell with other retinal neurons can be known and the retinal pathways along which visual information travels can be elucidated. Monochromatic stimuli produced from calibrated optical benches allow the measurement of rod and different classes of cone input in the responses of individual cells. Movable bar stimuli allow the characterization of receptive field properties in terms of a single number, the space constant, which relates to the electrical interactions with neighboring cells, and thus to anatomically observable gap junctions.

Major Findings:

I. A new bipolar cell type in the Rhesus monkey retina:

A bipolar cell type forming invaginating contacts with many cones was found by light & electron microscopy of Golgi preparations of the macaque (Maccaca mulatta) retina. This diffuse invaginating cone bipolar cell resembles, superficially, rod ("mop") bipolars, and so may correspond to Polyak's "brush" bipolar. However, it differs from rod bipolars in that its

dendrites are finer and they end in a single stratum containing cone pedicles in the outer plexiform layer (OPL). In the inner plexiform layer, its axon terminal is located sclerad (S_4) to those of rod bipolars (S_5), and also, is thinner, more branched and wider in span than rod bipolar axon terminals. Resectioning of Golgi-impregnated diffuse invaginating cone bipolars to study their connections in the OPL shows that their dendrites invaginate six to seven cone pedicles, and terminate as central elements at the ribbon synaptic complex. Although previously others have assumed that all the central elements at ribbon synapses of primate cones originated from midget bipolar dendrites, this is not the case as evidenced by this new cell type and its connections with cones. Thus, the primate retina has multiple (diffuse) and single (midget) cone-contacting bipolar cell pathways in both invaginating as well as flat varieties.

II. Regional variations in interreceptor contacts in primate retina:

Over the past several years physiological studies have demonstrated the functional importance of interreceptor contacts, which appear to allow electrical signals to spread among photoreceptors. Although it has been suggested that interreceptor contacts would improve signal-to-noise ratio, such contacts would appear disadvantageous for high visual acuity. We have been examining their occurrence in specialized regions of rhesus macaque retina, which increase in visual acuity from the periphery to the foveola.

Contacts between lateral surfaces of cone pedicles or between processes emanating from cone pedicles are characterized by a region of cytoplasmic density associated with plasma membranes separated by approximately 12 nm and an adjacent region of close membrane apposition where the intercellular space is 2-3 nm. These regions resemble the gap junctions described by Raviola and Gilula in thin sections and freeze-fracture replicas. Pedicle membrane appositions involving processes are most frequently observed and longest in peripheral retina, where many rod spherules intervene between cone pedicles. However, they are also found in central retina and on the foveal slope. Contacts between lateral surfaces of cone pedicles are most frequently found in central retina and on the foveal slope where pedicles are not widely separated.

Contacts between cone pedicles or their processes and rod spherules also involve regions of close membrane apposition adjacent to more widely separated membranes associated with cytoplasmic density and resemble gap junctions. These contacts are found in peripheral and central retina and on the foveal slope where rod spherules first appear.

Physiological studies in reptiles and amphibia have shown rod-rod junctions to be highly significant for the physiological properties of rods. In primates, regions where the plasma membranes of adjacent rod spherule are not separated by intervening glia have been reported but no specialized contacts between rod spherules have been described. We have found areas of specialized membrane apposition between rods where plasma membranes are

closely apposed and where one spherule often appears to invaginate the other. These regions are most frequently found in peripheral retina, where many rods are typically adjacent, and are seen less often in central retina, where the percentage of juxtaposed rods is lower.

In the foveola, which has not been previously described by electron microscopy in monkey, cone pedicles and their conducting fibers are widely separated and heavily ensheathed by glia, appearing to be effectively insulated from each other. No processes, which might provide a means of contact, have been observed to emanate from any pedicles within the foveola which belong to the narrow, centralmost cones of the foveola, those of the "central bouquet". The conducting fibers of these cones were found to be narrower than those of other cones, approximately $2/3$ the diameter, and thus it was possible to identify the conducting fibers of central bouquet cones and to trace them to their pedicles. The pedicles of the outermost cones of the central bouquet are found on the foveal slope where the ganglion cells are 2-3 deep, and in this region the striking difference in diameters of the conducting fibers and in thicknesses of glial ensheathment of these fibers may be compared between cones of the central bouquet and those outside this region. It seems appropriate to the high visual acuity of the foveola, that the centralmost cones of this region, those of the central bouquet, are effectively insulated from each other. The decrease in visual acuity associated with increasing distance from the foveola may well be associated with increased occurrence of interreceptor contacts.

III. Functional stratification of cone bipolar cell axons in the cat retina.

The level of axonal stratification of hyperpolarizing and depolarizing cone bipolars (hCB and dCB) in the cat retina has been examined with intracellular recording followed by marking with Procion or horseradish peroxidase. Of six dCB's all had axons branching in the middle of the inner plexiform layer (IPL) (s3, s4 of Cajal) in sublamina b. Cell bodies were found either high, in the horizontal cell layer, or in the middle of the inner nuclear layer (INL). Of seven hCB's, five had axonal terminals ending in sublamina a (the outer $1/3$ of the IPL), one arborized in sublamina b (in s3) and the last was incompletely stained. Cell bodies were found at all levels in the INL. Comparisons with Golgi-stained cells, elsewhere studied by electron microscopy, suggest that dCB's are the invaginating and hCB's the flat cone bipolar types. Receptive field properties of cone bipolars varied systematically with the level of axonal arborization. Cells branching in sublamina b had tiny receptive field centers (space constants of 20 to 100 μm), some with antagonistic surrounds. Cells arborizing in sublamina a had larger receptive fields (space constants of 100 to 300 μm), some as large as receptive fields found in horizontal cell bodies. Thus electrical coupling may exist between bipolar cells branching in sublamina a. No antagonistic surrounds were observed in this layer. Spectrally, cone bipolars occurred in two varieties. Six had mixed rod and cone input similar to the responses of cones and horizontal cell bodies. Five were dominated by rod input. The responses of dCB's of the latter type closely resembled those of AII (rod) amacrine cells, suggesting a

functional interconnection.

IV. Responses of pigeon horizontal cells:

Physiology of the in vitro, perfused pigeon retina: The pigeon retina is complex in its cellular and synaptic organization, and this anatomical complexity is displayed physiologically at the ganglion cell level. Yet within this complex system, the inner and outer plexiform layers are highly organized especially with respect to their stratification due primarily to the highly stereotyped morphologies of neuronal types which contribute processes to these synaptic layers. Our interest in the physiology of horizontal cells is based on the psychophysical observations that pigeons display extremely good color discrimination and, that anatomically six spectral classes of cones and their connections with horizontal cells have been identified. Previously, we reported work aimed at maintaining a viable, in vitro preparation necessary to obtain successful intracellular recordings. This goal has been accomplished, and we have proceeded to the point of being able to regularly obtain intracellular responses from the very small horizontal cells. The responses thus far obtained show a sustained hyperpolarization to flashed slits of light with a fast transient depolarization at the offset of the stimulus. Although the sustained hyperpolarizing component is similar to that reported in many other vertebrate horizontal cells, the fast transient depolarization at stimulus off is quite different from previously reported horizontal cell responses. Work is in progress to identify the cause of this off effect and to use chromatic stimuli to better understand the role of horizontal cells in color vision. By the use of appropriate intracellular stains we hope to relate the physiology to the anatomical connections.

Significance to Biomedical Research and the Program of the Institute:

In diagnosing and treating the diseases of the eye it should prove useful to understand retinal function at the cellular level and the pathways through which visual signals travel and are processed. In this regard it is interesting that our repertoire of intracellularly studied and stained neurons now includes several from cats afflicted with central retinal degeneration (CRD). These are not in sufficient quantities to draw definite conclusions concerning modifications of retinal pathways. Our recent discovery in the cat retina of the extreme sensitivity of the ERG b-wave to the spatial pattern of the stimulus has recently been demonstrated also in humans by Zrenner and Diehl and may have clinical value. Many disease states involve dysfunction at the cellular level and treatments have as their targets particular classes of cells. A knowledge of what classes of neurons the retina contains and what their physiological properties and roles in vision may be provides a necessary substrate for interpreting and treating retinal dysfunction.

Proposed Course: This project will be continued along lines indicated in the project description. Emphasis will be given to neurons participating in the inner and outer plexiform layers of primates and cats. Comparisons will continue to be made between homologous cell types in the retinas of different species using the Golgi staining technique. Particular emphasis

will be given to interreceptor contacts in primates. Existing data supplemented by additional data currently being obtained on bipolar and amacrine cells in the cat retina will be organized and made ready for publication. The interrelationships of amacrine, bipolar and ganglion cells in the cat IPL will be further studied.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications:

Nelson R, Zrenner E, Gouras P: Patterned stimuli reveal spatial organization in the electroretinogram, in Tazawa Y (ed): Proceedings of the XVIth ISCEV Symposium. Morioka, Jpn J Ophthalmol, 1979, pp 161-169.

Gouras P, Zrenner E: Enhancement of flicker by color opponent mechanisms. Science 205:587-589, 1979.

Zrenner, E, Gouras P: Blue cones of the cat produce a rod-like electroretinogram. Invest Ophthalmol Vis Sci 18:1076-1081, 1979.

Kolb H, Mariani A, Gallego A: A second type of horizontal cell in monkey retina. J Comp Neurol 189:31-44, 1980.

Nelson R, Kolb H, Robinson M, Mariani A: Neural circuitry of the cat retina: Cone pathways to ganglion cells, in Information Processing in the Retina: Suppl. to Proceedings of the XXVIII International Congress of Physiological Sciences. 1980 (in press)

Kolb H, Nelson R: Morphology and circuitry of some amacrine cells in the cat retina, in Information Processing in the Retina: Suppl. to the Proceedings of the XXVII International Congress of Physiological Sciences. 1980 (in press).

Nelson RF: Functional stratification of cone bipolar cell axons in the cat retina. Invest Ophthalmol Vis Sci 19(ARVO Suppl):130, 1980.

Dickinson-Nelson AD, Nelson RF: Regional variations in interreceptor contacts in primate retina. Invest Ophthalmol Vis Sci 19(ARVO Suppl): 71, 1980.

Mariani AP: A "diffuse" invaginating cone bipolar cell in primate retina. Invest Ophthalmol Vis Sci 19(ARVO Suppl):71, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00066-03 LVR
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Neurotransmitter Chemistry of Retinal Neurons		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Barbara-Anne Battelle Ph.D. Senior Staff Fellow LVR NEI Other: None		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Retinal and Corneal Metabolism		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Studies are underway to identify <u>chemical neurotransmitters in photoreceptor cells</u> and other <u>retinal neurons</u> , and to examine the role of chemical neurotransmitters in <u>processing visual information</u> . Particular attention is given to the role of <u>biogenic amines in visual function</u> . Two systems are currently being investigated: (1) the relatively simple visual system of <u>Limulus polyphemus</u> and (2) dissociated <u>mammalian retinal neurons grown in monolayer culture</u> . Biochemical (<u>high voltage electrophoresis</u> and <u>high performance liquid chromatography coupled with electrochemical detection</u>) and anatomical (<u>autoradiography</u>) techniques are employed to identify and localize chemical neurotransmitter systems present in these preparations. <u>Electrophysiological techniques</u> are being developed to study the interactions of isolated retinal neurons.		

Project Description:

Objectives: The general aim of my research is to identify neurotransmitters used by retinal neurons and to examine neurochemical mechanisms involved in processing visual information.

Methods Employed: High voltage electrophoresis and other chromatographic techniques are employed to study synthesis, accumulation and metabolism of putative neurotransmitters from radioactively labeled precursors. Sensitive enzymatic assays and high performance liquid chromatography coupled with electrochemical detection are used to measure endogenous levels of amines. Sites of synthesis of putative neurotransmitters are localized using autoradiography. Two preparations are being studied: (1) the relatively simple visual system of Limulus polyphemus and (2) dissociated mammalian retinal neurons grown in monolayer culture.

Major Findings: (1) Neurotransmitters in the visual system of Limulus polyphemus. Evidence gathered in this laboratory suggests that the biogenic amine octopamine may be an important neurotransmitter in the visual system of Limulus. Synthesis of GABA, acetylcholine, dopamine, octopamine and serotonin was studied in various tissues of the Limulus visual system. Among these neurotransmitter candidates, only octopamine was synthesized to any significant extent in the eyes. High levels of endogenous octopamine were also found in Limulus eyes and optic ganglia. In order to localize sites of octopamine synthesis and storage, autoradiography was used. Results of these experiments show convincingly that octopamine is synthesized and present in a population of small, efferent fibers that project from the CNS to the ventral photoreceptor cells. These efferent fibers are believed to innervate photoreceptor cells and modify their structure and function. Other experiments which revealed the presence of octopamine receptors in Limulus eyes strengthen the hypothesis that octopamine is a neurotransmitter in these tissues. (2) Growth and differentiation of mammalian retinal neurons in monolayer culture. Procedures have been developed which permit the growth and differentiation of mammalian retinal neurons in monolayer culture with minimum contamination from non-neuronal cells. Cells dissociated from retinas of new-born rats survive up to two weeks in culture and elaborate an extensive network of processes. Some cells aggregate during the first 5 days; however, others remain solitary. Solitary cells can be characterized into several different cell types on the basis of their overall morphology. Cultured retinal cells also synthesize the retinal neurotransmitter candidates GABA, acetylcholine and dopamine from radioactive precursors indicating that they develop and retain neuron-specific functions:

Significance to Biomedical Research and the Program of the Institute:

- (1) Many retinal diseases result from abnormal functioning of photoreceptor cells. Identification of the biogenic amine octopamine within fibers that influence photoreceptor cell function will allow a detailed biochemical study of mechanisms underlying the control of some aspects photoreceptor cell function such as diurnal changes in sensitivity and membrane reorganization. From these studies we may gain an understanding of causes underlying abnormal photoreceptor cell function as well.
- (2) The development of a system for growing mammalian retinal neurons in monolayer culture provides a critical tool for the study of the interactions of identified retinal neurons. This system will be important in elucidating mechanisms of both normal and abnormal retinal functions.

Proposed Course: (1) Studies of the role of octopamine in the control of photoreceptor cell function will continue. Octopamine receptors will be located and characterized biochemically, and the physiological stimuli of octopamine release will be identified. (2) The development of chemical neurotransmitter systems in the retinal monolayer cultures will be studied biochemically and cells responsible for the synthesis of specific neurotransmitter candidates will be identified.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications:

Battelle BA, LaVail MM: Protein synthesis in retinas of rats with inherited retinal dystrophy. Exp Eye Res (in press).

Battelle BA: Neurotransmitter candidates in the visual system of *Limulus polyphemus*: synthesis and distribution of octopamine. Vision Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00148-07 LVR																		
PERIOD COVERED October 1, 1979, to September 30, 1980																				
TITLE OF PROJECT (80 characters or less) Cyclic Nucleotides and Vision																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">Gerald J. Chader</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Research Chemist</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td></td> <td>Y.P. Liu</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>R. Theodore Fletcher</td> <td>M.S.</td> <td>Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI		Y.P. Liu	Ph.D.	Staff Fellow	LVR	NEI	Other:	R. Theodore Fletcher	M.S.	Chemist	LVR	NEI
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	Y.P. Liu	Ph.D.	Staff Fellow	LVR	NEI															
Other:	R. Theodore Fletcher	M.S.	Chemist	LVR	NEI															
COOPERATING UNITS (if any) Clinical Pharmacol. Branch, NCI, Lab. Chem. Pharmacol. NHLBI, Section Ophthalmol., School of Vet. Medicine, Univ. Penn., Phila., PA																				
LAB/BRANCH Laboratory of Vision Research																				
SECTION Section on Retinal and Corneal Metabolism																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																				
TOTAL MANYEARS: 1.75	PROFESSIONAL: 0.75	OTHER: 1.0																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Cyclic nucleotides</u>, especially <u>cyclic GMP</u>, are important in the <u>visual process</u> and in other functions of the <u>retina</u> and <u>pigment epithelium</u>. Deficiencies in the enzymes of cyclic nucleotide metabolism may also be responsible for diseases related to retinal dysfunction. In a dog model for <u>retinitis pigmentosa</u> it appears that a switch in cyclic GMP <u>phosphodiesterase</u> (PDE) fails to occur during development in retinas of affected animals. This, coupled with low levels of calmodulin in affected retinas, results in abnormally high cyclic GMP concentration and photoreceptor degeneration. <u>Protein kinase</u> activity is also important in the photoreceptor and can be controlled by cation and nucleotide concentration as well as several other modulators naturally present in outer segments. </p>																				

Project Description:

Objectives: To study the role of cyclic nucleotides and the enzymes of cyclic nucleotide metabolism in normal vision and in retinal disease.

Methods Employed: Retinas from test animals are dissected; photo-receptor units are isolated by sucrose density gradient ultracentrifugation and the activities of the enzymes of cyclic nucleotide metabolism and protein kinase are assayed by standard techniques. Cyclic nucleotide concentrations are measured by immunochemical titration after initial purification by column chromatography.

Major Findings: (1) A defect in enzyme activity in the retinas of Irish setter dogs with inherited retinal degeneration has been found. The phosphodiesterase (PDE) enzyme which metabolizes cyclic GMP is deficient in affected animals. Moreover, the concentration of calmodulin, the protein activator of the PDE enzyme is low in affected retinas. This enzymatic deficit apparently leads to the high cyclic GMP levels that are characteristic of this early onset disease. In contrast to the situation in the setter, cyclic nucleotide levels are "normal" in other canine models of retinitis pigmentosa such as in the miniature poodle. (2) Retinal photo-receptor membranes exhibit high GTP-kinase as well as ATP-kinase activity. Fluxes in cation and metabolite concentrations as well as availability of ATP and GTP affect kinase activity in vitro and could exert a major influence on kinase activity in vivo affording the possibility of differential phosphorylation under various physiological conditions.

Significance to Biomedical Research and the Program of the Institute:

(1) If the finding concerning the abnormality in the Irish setter dog retina is correct and is found to be similar in humans, the cause of at least one form of retinitis pigmentosa will be understood. This then could subsequently lead to a rational mode of treatment of the disease. Since affected retinas of the miniature poodle do not demonstrate an abnormality in cyclic GMP concentration, we conclude that there may be several types of degeneration of varying biochemical etiologies each of which will have to be investigated separately. (2) Understanding of the control of protein kinase activity in the outer segment will greatly increase our knowledge as to the basic functioning of the photoreceptor unit.

Proposed Course: (1) Studies on cyclic nucleotides and the PDE enzyme will continue in other animal models of retinal degeneration. We will also begin a regimen of therapy in the affected dogs in hopes of stopping or at least slowing down the course of the disease. (2) Similarly, studies on protein kinase activity will continue to further define the control mechanisms for this enzyme system.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Liu L, Krishna G, Aguirre G, Chader GJ: Cyclic GMP phosphodiesterase Activator: Involvement in a hereditary retinal degeneration. Nature 280:62-64, 1979.

Chader GJ, Liu Y, O'Brien P, Fletcher RT, Krishna G, Aguirre G, Farber D, Lolley R: Cyclic GMP phosphodiesterase activator: involvement in a hereditary retinal degeneration. Neurochemistry 1:441-458, 1980.

Tamai M, Chader GJ: The early appearance of disc shedding in the rat retina. Invest Ophthalmol Vis Sci 18:913-917, 1979.

Newsome D, Fletcher RT, Chader GJ: Cyclic nucleotides vary by area in the retina and pigmented epithelium of the human and monkey. Invest Ophthalmol Vis Sci (in press).

Chader GJ, Fletcher RT, Russell P, Krishna G: Differential control of protein kinase activities of the retinal photoreceptor: cation effects on phosphorylation by ATP and GTP. Biochemistry (in press).

Chader GJ, Fletcher RT, Krishna G: Guanine nucleotides: importance in visual process of the rod outer segment. Yale J Biol Med (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00041 02 LVR																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Retina Lipid Metabolism: Correlation with a Circadian Rhythm and Effect of Light																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">Peter Dudley</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 10%;">LVR</td> <td style="width: 15%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Paul J. O'Brien</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Martin Zatz</td> <td>M.D.</td> <td>Research Chemist</td> <td>LCS</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Sanford Markey</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LCS</td> <td>NIMH</td> </tr> </table>			PI:	Peter Dudley	Ph.D.	Staff Fellow	LVR	NEI	Other:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI		Martin Zatz	M.D.	Research Chemist	LCS	NIMH		Sanford Markey	Ph.D.	Research Chemist	LCS	NIMH
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COOPERATING UNITS (if any) None																										
LAB/BRANCH Laboratory of Vision Research																										
SECTION Section on Retinal and Corneal Metabolism																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.4	OTHER: 0																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) The shedding of rat rod outer segment (ROS) discs, and the process of phagocytosis, is a <u>circadian</u> phenomenon characterized morphologically by the engulfment of ROS discs by the pigment epithelium. This process occurs over a period of a few hours beginning two hours after the start of the light period. The daily synthesis of new retinal photoreceptor membranes also occurs over a period of several hours, peaking at 4 p.m., three hours before the beginning of the dark period. This event has been detected by incubation of retinas with <u>labeled glycerol</u> for 45 minutes followed by separation of phospholipid classes by thin-layer chromatography. S-adenosyl methionine (SAM) is a methyl donor which <u>transmethylates</u> phosphatidyl ethanolamine to form phosphatidyl choline. This event occurs in bovine ROS membranes and may be related to alteration of membrane fluidity. Besides phospholipids, fatty acid methyl esters and prostaglandin-like neutral lipids are transmethylated by SAM, the reaction being sensitive to light and perhaps involved in photo-transductions.																										

Project Description:

Objectives: Biochemical events associated with circadian synthesis of vertebrate photoreceptor membranes have not been documented. The objectives of this project are (1) to determine if labeling of retina membrane phospholipids in the rat occurs at a specific time on a daily basis, (2) to ascertain whether this is a circadian phenomenon, and (3) to determine the significance of lipid methylation in membrane synthesis (polar lipids) and membrane transduction (neutral lipids).

Methods Employed: Ordinary biochemical techniques were employed such as incubation of retinas, extraction of lipids and separation of neutral and polar lipids by thin layer chromatography.

Major Findings: Increased phospholipid synthesis occurred in rat retina during the daylight hours, peaking at 4 p.m. for animals maintained on a 7 a.m.: 7 p.m. light schedule. Rat retinas incubated in vitro with (³H)-methionine showed the production of methylated phospholipids as well as neutral lipids. The neutral lipids which became methylated were (1) fatty acids and (2) a prostaglandin-like lipid. Methylated neutral lipid production was enzymatic, stimulated by phospholipases that cleave fatty acids from phospholipids, and was increased in the light over the dark.

Significance to Biomedical Research and the Program of the Institute: Specific biochemical events associated with the circadian synthesis of photoreceptor membranes have not been demonstrated. The knowledge acquired from the study of events surrounding insertion of membrane components into newly formed photoreceptor membranes may give a better understanding of how this process is controlled.

Changes in the methylation of membrane lipids as a result of light stimulation may be at the core of understanding the mechanism by which photoreceptor membranes respond to light.

Proposed Course: The circadian nature of phospholipid synthesis will be examined by incubating retinas from animals kept in constant darkness with known phospholipid precursors. The light cycle will be manipulated to determine if the peak of synthesis can be shifted. Pharmacological experiments will be used to determine what chemical agents control the circadian synthesis of retinal membranes.

NEI Research Program: Retina and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00106-01 LVR												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Cyclic Nucleotides and Vision														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">Yung-Pin Liu</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 25%;">Senior Staff Fellow</td> <td style="width: 15%;">LVR</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Dr. Gerald Chader</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table> Co-step Program: Brenda Waller (January 2, 1980 to August, 1980) Howard University Joy Dixon (May 19, 1980 to August, 1980) Howard University			PI:	Yung-Pin Liu	Ph.D.	Senior Staff Fellow	LVR	NEI	Other:	Dr. Gerald Chader	Ph.D.	Research Chemist	LVR	NEI
PI:	Yung-Pin Liu	Ph.D.	Senior Staff Fellow	LVR	NEI									
Other:	Dr. Gerald Chader	Ph.D.	Research Chemist	LVR	NEI									
COOPERATING UNITS (if any) Dept. Ophthalmology, Univ. Penn. School Veterinary Medicine, Phila., PA Dept. Ophthalmology, Univ. Illinois Medical School, Chicago, IL														
LAB/BRANCH Laboratory of Vision Research														
SECTION Section on Retinal and Corneal Metabolism														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER: 0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) I. Low levels of <u>cyclic GMP phosphodiesterase</u> (PDE) coupled with insufficient amount of <u>calmodulin</u> in retinas of Irish setter dogs with inherited degeneration could be responsible for the high concentrations of cyclic GMP presented in these degenerated dog retinas. II. The relationship of the <u>retinal degeneration</u> and cyclic GMP PDE and calmodulin was also investigated in hereditary retinopathy in collies. Cyclic GMP PDE levels rose as outer segments developed in the control dogs, while in the affected animals, cyclic GMP PDE levels were generally low, and the molecular form of cyclic GMP PDE is Ca^{++} and <u>calmodulin-independent</u> . III. In bovine retina extracts, cyclic CMP PDE may be a separate enzyme distinguishable from cyclic AMP PDE and cyclic GMP PDE on the basis of differential inhibition by <u>PDE inhibitors</u> .														

Project Description:

Objectives: To characterize the properties of enzymes and calmodulin hydrolyzing cyclic nucleotides in normal and in diseased eye tissues.

Methods Employed: Cyclic nucleotide PDE and calmodulin are assayed with radiolabeled substrates and ion-exchange resins. Retinas from dogs and bovines are dissected. Sucrose density gradient ultracentrifugation was performed to isolate photoreceptor units.

Major Findings: (1) Low specific activity of cyclic GMP PDE and insufficient calmodulin in the retinas of Irish setter dogs with inherited retinal degeneration have been found. This defect in enzyme and calmodulin apparently was the cause for the high cyclic GMP levels in the retinas of affected Irish setter dogs. (2) We extended our biochemical studies to another model, hereditary retinopathy collies. The levels of cyclic GMP were higher in affected than in control retinas particularly between 20 and 45 days, but cyclic AMP levels in affected retinas remained similar to control levels. In the carrier control retina, cyclic GMP PDE levels rose as outer segments developed, while in the affected animals cyclic GMP PDE levels were generally low. In addition, the type of PDE present in the affected collies is Ca^{++} and calmodulin-independent. (3) We have studied the effects of various agents on the cyclic CMP, cyclic AMP and cyclic GMP PDE of bovine retina extracts. The results suggest that cyclic CMP PDE may be a separate enzyme distinguishable from cyclic AMP PDE and cyclic GMP PDE on the basis of differential inhibition by inosine 5'-monophosphate, 3-isobutyl-1-methylxanthine, papaverine, and theophylline, and that it may play important roles in regulating pyrimidine cyclic nucleotide.

Significance to Biomedical Research and the Program of the Institute: Although cyclic GMP levels are high in both retinas of Irish setter dogs and collies, it shows that calmodulin may directly associate with only the affected Irish setter dogs. Thus, there may be more than one type of degeneration of varying biochemical etiologies in dogs. With the knowledge of the defect cyclic GMP metabolism in Irish setter and access to purified calmodulin we now have an opportunity to possibly increase retinal PDE activity in vivo and perhaps to slow or even halt the progress of the disease. Understanding of the role of cyclic CMP PDE in regulating pyrimidine cyclic nucleotide in the outer segment will greatly increase our knowledge as to the basic functioning of the photoreceptor unit.

Proposed Course: (1) With the availability of degenerative Irish setter dogs intraocular injections of calmodulin will be performed in hopes of stopping or at least slowing down the course of the disease. (2) Studies on PDE and calmodulin will continue with retinal degeneration mice. (3) Isolation, purification and characterization of cyclic GMP PDE will continue to further understand the control mechanisms for this enzyme system.

NEI Research Program: Retinal and Choroidal Diseases--Developmental
and Hereditary Disorders

Publications:

Liu YP, Krishna G, Aguirre G, Chader G: Inherited retinal degeneration. Involvement of cyclic GMP phosphodiesterase and protein activator. Nature 280:62-64, 1979.

Liang CM, Liu YP, Chabner BA: Isolation and identification of a small molecular weight inhibitor of cyclic nucleotide phosphodiesterase from bovine brain. Biochim Biophys Acta 571:63-69, 1979.

Liang CM, Liu YP, Chabner BA: Modes of action of hypoxanthine, inosine and inosine 5'-monophosphate on cyclic nucleotide phosphodiesterase bovine brain. Biochem Pharmacol 29:277-282, 1980.

Chader G, Liu Y, O'Brien P, Fletcher R, Krishna G, Aguirre G, Farber D, Lolley R: Cyclic GMP phosphodiesterase activator: involvement in a hereditary retinal degeneration. Neurochemistry 1:441-458, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00068-03 LVR												
PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Physiology of the Pigment Epithelium														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">Eileen Masterson</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 25%;">Staff Fellow</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Gerald J. Chader</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Eileen Masterson	Ph.D.	Staff Fellow	LVR	NEI	Other:	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI
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Other:	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI									
COOPERATING UNITS (if any) None														
LAB/BRANCH Laboratory of Vision Research														
SECTION Section on Retinal and Corneal Metabolism														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0												
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The relationship between <u>pigment epithelial cell</u> metabolism and phagocytosis was studied. The intact functioning of the <u>TCA cycle</u> and <u>cytochrome system</u> is necessary for these cells to optimally <u>phagocytize</u> rod outer segments.														

Project Description:

Objectives: To characterize pigment epithelial metabolism and its relationship to the physiologic function of this cell.

Methods Employed: Pigment epithelial cell cultures were maintained using standard tissue culture techniques. Cells at confluency were studied as to their ability to phagocytize rod outer segments under various conditions, including incubation with metabolic poisons of several types.

Major Findings: Characterization of hexose transport using the non-metabolizable sugar 3-O-methyl-D-glucose demonstrated that cultured pigment epithelial cells have a facilitated diffusion system of uptake for this molecule. Phagocytosis did not stimulate glucose transport by these cells. Phagocytosis of rod outer segments was markedly inhibited by glucose depletion of the incubation media and by two inhibitors of the tricarboxylic acid cytochrome system (malonate and dinitrophenol). Lactate production increased in phagocytizing cells, but the hexose monophosphate shunt was not stimulated. The data support the conclusion that the tricarboxylic acid cycle and its associated cytochrome system play an important role as an energy source for phagocytosis. Compromise of this energy source rapidly leads to depression of the phagocytic process.

Significance to Biomedical Research and the Program of the Institute: Failure of the pigment epithelium to phagocytize outer segments will lead to retinal disease. It is believed that such a failure occurs in the human disease retinitis pigmentosa. These studies indicate that compromise of the metabolic machinery of the pigment epithelial cell could lead to a block in phagocytosis, and thus retinal diseases.

Proposed Course: The metabolic capability of the pigment epithelial cell will continue to be investigated. Specifically, what molecules are used for energy sources by these cells will be characterized, and the metabolic fate of glucose studied. In addition, the regulation of phagocytosis by these cells will be investigated.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Israel P, Masterson E, Goldman AI, Wiggert B, Chader GJ: Retinal epithelial cell differentiation: Influence of culture medium in vitro. Invest Ophthalmol Vis Sci (In Press).

Masterson E, Goldman AI, Chader GJ: Phagocytosis of rod outer segments by cultured epithelial cells. Vis Res (In Press).

Masterson E, Chader GJ: Pigment epithelial cells in culture:
Metabolic pathways required for phagocytosis. Invest Ophthalmol
Vis Sci (In Press).

Masterson E, Chader GJ: Characterization of glucose transport by
cultured chick pigmented epithelium. Exp Eye Res (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00016-13 LVR																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) The Biochemistry of Normal and Dystrophic Retinas																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
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COOPERATING UNITS (if any) School of Veterinary Medicine, University of Pennsylvania Veterans Administration Hospital, Sepulveda, CA																										
LAB/BRANCH Laboratory of Vision Research																										
SECTION Section on Retinal and Corneal Metabolism																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3																								
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SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Opsin synthesis</u> was measured in <u>Irish setters</u> affected with a <u>recessively inherited retinal dysplasia</u>. Photoreceptors fail to differentiate normally and <u>cyclic GMP</u> levels are greatly elevated during differentiation prior to the time when the photoreceptors degenerate. However, opsin synthesis was found to occur at a normal rate at a time when cyclic GMP levels had already risen substantially but morphological changes were not yet evident. Thus, no generalized abnormality in protein synthesis was detected which suggests that the elevation in cyclic GMP, caused by a defect in the synthesis of a specific phosphodiesterase, may represent the primary biochemical abnormality. </p>																										

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is to examine biochemical events unique to the retina, especially the synthesis of photoreceptor membrane components, in the retinas of vertebrates which can be affected by inherited retinal degenerations.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas, cell fractionation, isolation of rod outer segments by density gradient centrifugation, detergent extraction and purification of rhodopsin by column chromatography and gel electrophoresis.

Major Findings: The synthesis of membrane proteins, particularly opsin, was found to occur at an identical rate during the early stages of photoreceptor outer segment development in both normal controls and in Irish setters affected with a recessively inherited retinal dysplasia. During this same period, cyclic GMP levels rose to abnormally high levels, but no morphological abnormalities could be detected until a later time.

Significance to Biomedical Research and the Program of the Institute: The elevation of cyclic GMP in photoreceptors of affected setters is caused by a defect in the production of a specific phosphodiesterase. This study shows that no generalized defect in protein synthesis occurs in the photoreceptors and thus points to a specific defect in the synthesis of the phosphodiesterase which may represent the primary defect in this inherited rod-cone dysplasia. A comparable defect could produce retinitis pigmentosa in humans.

Proposed Course: Both Irish setters and miniature poodles with inherited retinal degenerations will be studied further to search for defects in the synthesis of photoreceptor membrane components, particularly glycoproteins.

NEI Research Program: Retinal and Choroidal Diseases--
Developmental and Hereditary Disorders

Publications:

Chader G, Liu Y, O'Brien P, Fletcher R, Krishna G, Aguirre G, Farber D, Lolley R: Cyclic GMP phosphodiesterase activator: involvement in a hereditary retinal degeneration. Neurochemistry International 1:441-458, 1980.

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PERIOD COVERED October 1, 1979, to September 30, 1980														
TITLE OF PROJECT (80 characters or less) The Cell Biology of the Vertebrate Retina														
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COOPERATING UNITS (if any) None														
LAB/BRANCH Laboratory of Vision Research														
SECTION Section on Retinal and Corneal Metabolism														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.7	OTHER: 0.7												
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) Biochemical correlates to <u>circadian photoreceptor outer segment shedding</u> were sought. Opsin synthesis as a function of the ambient light cycle was followed by studying radioactive <u>leucine</u> and <u>glucosamine</u> incorporation in vitro. Both precursors showed a <u>diurnal rhythm</u> of incorporation with a maximum occurring during the early morning hours prior to light onset. Glucosamine exhibited a more pronounced peak than leucine. This rhythm persisted when light-entrained animals were maintained in <u>constant darkness</u> indicating that opsin synthesis is a <u>circadian rhythm</u> that may be related to circadian photoreceptor shedding.														

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. In the process of renewal of photoreceptor outer segment disc membranes, rhodopsin, a glycoprotein, must be transported from the inner segment and incorporated into disc membranes with a specific orientation in space. This project was designed to determine where and when sugars are added to the polypeptide and what role they play in the transport and assembly of rhodopsin into disc membranes and in the process of shedding and phagocytosis of disc membranes. In addition, biochemical correlates to circadian photoreceptor shedding will be sought, particularly in relation to glycoprotein synthesis and function.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas with radioactive precursors, cell fractionation, SDS gel electrophoresis, and scintillation counting.

Major Findings: Radioactive leucine and glucosamine were incorporated into opsin in rat retinas incubated in vitro at various times throughout a normal 12 hour light and 12 hour dark cycle. The rate of opsin synthesis varied with the phase of the cycle. The incorporation was elevated during the dark hours, especially the 4 hours preceding the onset of light, after which synthesis fell rapidly. Glucosamine exhibited a greater differential between night and day than did leucine. Constant darkness, which permits the daily, circadian photoreceptor shedding peak to appear, also supported the elevated glucosamine incorporation during the night hours.

The rat retina also was found to incorporate galactose into opsin in vitro, just as bovine and frog retinas do.

Significance to Biomedical Research and the Program of the Institute: There are two known prerequisites to circadian photoreceptor shedding in the rat retina: (1) at least two hours of darkness and (2) approximately a 24 hour interval between shedding events. It is presumed that this reflects two hours of light-sensitive reactions and 24 hours of preparatory, possibly biosynthetic, reactions. These results suggest that opsin synthesis describes a 24 hour cycle that may in fact, be a circadian oscillator that controls shedding. Identification of unique events such as this provides an opportunity to examine potential sites of retinal-specific lesions in retinas with inherited degenerative disorders.

Proposed Course: The response of leucine and glucosamine incorporation into opsin to such factors as constant light, constant darkness or shifts in the light cycle will be examined. All of these factors have profound affects on the circadian shedding process and will be used to

determine which biochemical events correlate with the shedding rhythm. In addition galactose incorporation will be studied as a function of the light cycle to determine the optimal conditions for possible autoradiography.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

O'Brien PJ: Glycosyl transfer to bovine rhodopsin. In Gregory JD and Jeanloz RW (eds): Glycoconjugate Research. New York, Academic Press, 1979, Vol. 2, pp 729-31.

Goldman AJ, Teirstein PS, O'Brien PJ: The role of ambient lighting in circadian disc shedding in the rod outer segment of the rat retina. Invest Ophthalmol Vis Sci (in press).

Teirstein PS, Goldman AJ, O'Brien PJ: Evidence for both local and central regulation of rat rod outer segment disc shedding. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00070-03 LVR																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Vitamin A and Ocular Tissues																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
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COOPERATING UNITS (if any) Howe Laboratory of Ophthalmology, Harvard Medical School																										
LAB/BRANCH Laboratory of Vision Research																										
SECTION Section on Retinal and Corneal Metabolism																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 2.2	PROFESSIONAL: 1.4	OTHER: 0.8																								
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SUMMARY OF WORK (200 words or less - underline keywords) Synthetic <u>analogs of retinol</u> and <u>retinoic acid</u> were used in studies with Y-79 <u>human retinoblastoma cells</u> . Both ³ H-13 cis - retinoic acid and ³ H-5,6 epoxy-retinoic acid were bound to CRABP in these cells. ¹⁴ C - <u>retinyl palmitate</u> and ¹⁴ C-palmitic acid were bound to a <u>6S protein</u> in <u>pigment epithelium - choroid cytosol</u> . There was no detectable binding of ¹⁴ C-retinyl palmitate in retinal cytosol. <u>Gel filtration studies</u> indicate that a protein found in bovine brain cytosol which sediments at 7S and binds ³ H-retinol is apparently not the same as the soluble <u>7S retinol binding protein</u> found in <u>bovine retina</u> .																										

Project Description:

Objectives: To elucidate the mechanism of action of retinoids in ocular tissues.

Methods Employed: Sucrose density gradient centrifugation, autoradiography, gel filtration, thin layer chromatography, polyacrylamide gel electrophoresis, and isoelectric focusing were employed in studies of cellular retinoid binding proteins and vitamin A metabolism.

Major Findings: Retinoblastoma Cells

Synthetic analogs of retinol and retinoic acid were used in studies with Y-79 human retinoblastoma cells grown in tissue culture. Both ³H-13-cis retinoic acid and ³H-5,6 - epoxyretinoic acid were bound to CRABP (cellular Retinoic Acid Binding Protein) after incubation with retinoblastoma cells. Several unlabeled analogs of retinol and retinoic acid competed with ³H-retinol and ³H-retinoic acid for binding to CRBP (Cellular Retinol Binding Protein) and CRABP respectively. Autoradiographic studies of retinoblastoma cells incubated with ³H-13-cis retinoic acid revealed no preferential nuclear labeling as was observed after incubation of the cells with ³H-retinoic acid.

Retinyl Ester Binding

In studies using 11-12 day chick pigment epithelium-choroid cytosol, both ¹⁴C-retinyl palmitate and ¹⁴C-palmitic acid were observed to be bound at a peak sedimenting at about 6S on 5-20% sucrose gradients. Thin layer chromatography of extracts of this 6S peak following incubation of cytosol with ¹⁴C-retinyl palmitate demonstrated that both ¹⁴C-retinyl palmitate and ¹⁴C-palmitic acid were bound in this peak. After incubation with ¹⁴C-palmitic acid only ¹⁴C-palmitic acid was detected in the 6S peak. There was no detectable binding of ¹⁴C-retinyl palmitate in chick or bovine retinal cytosol. ¹⁴C-palmitic acid was found in peak sedimenting at 6S and 2S, however.

Transport Protein

Gel filtration studies of 7S cytosol proteins which specifically bind ³H-retinol in bovine cerebral cortex and retina revealed that these proteins are not identical in that the brain protein has a molecular weight of at least 1,500,000 whereas the retina protein has a molecular weight of between 600,000 and 1,500,000.

Significance to Biomedical Research and the Program of the Institute: In addition to its special role in the visual process, vitamin A is essential for the normal growth and differentiation of many tissues, particularly those of epithelial origin such as the retina. It is thus important to elucidate its mechanism of action and the role of cellular retinoid binding proteins in mediating this action in order to better

understand how ocular diseases related to vitamin A metabolism may be prevented or treated.

Proposed Course: Work will be continued on the role of cellular retinoid binding proteins and on the metabolism of retinoids in both fresh ocular tissues and in cells grown in tissue culture.

NEI Research Program: Retinal and Chorodial Diseases--Visual Cells and Pigment Epithelium

Publications:

Russell P, Wiggert B, Derr J, Albert D, Craft J, Chader GJ: Nuclear uptake of retinoids: Autoradiographic evidence and metabolic conversions in retinoblastoma cells in vitro. J Neurochem 34:1557-1560, 1980.

Wiggert B, Chader GJ: Cytosol binding of retinyl palmitate and palmitic acid in pigment epithelium and retina. Proceedings of the NY Acad of Sci (in press).

Chader GJ, Wiggert B, Russell P, Tanaka M: Retinoid binding proteins of retina and retinoblastoma cells in culture. Proc of the NY Acad of Sci (in press).

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